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INFLAMMATORY CYSTS AND CANCER OF THE BREAST

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FOR many years discussions have centred around the question of the association of cancer to inflammation, and from the various viewpoints of the subject there have developed many outspoken opinions and some theories. By some it has been shown, and to themselves conclusively proved, that inflammation was an important aetiological factor in the production of cancer, others found that all gradations could be observed between chronic inflammatory reactions and beginning cancers; while some again believed the inflammation to be only a coincident reaction in the cancerous process. The substance of each of these views has been expressed in the early history of scientific medicine, and since then there has been a constant reiteration of the same opinions; but we must admit that only recently has any satisfactory demonstration of a relationship been forthcoming. Even much of the evidence of this association which is offered to-day must be discarded.

In an excellent paper, in 1877, Friedlander discusses the occurrence of a typical epithelial growth, both in connexion with inflammation and with cancer. His observations show that the healing process of many epithelial structures is accompanied by proliferative changes which, as processes, are atypical, and in which, too, atypical cells are found. The skinning over of indolent ulcers gives rise to unusual activity on the part of the surrounding epithelium. Active mitosis leads to a thin film of epithelial cells which stretch inwards from the border, while larger islands of epithelial cells appear at some distance from the margin. In each of these areas the epithelial cells have taken on an unusual tendency to grow, and although a few columns of cells may be found dipping into the granulation tissue, the tendency is for the growth to remain on the surface. In this temporary, new rôle of activity, the epithelial cells alter their appearance very much. It is observed that the cells show little tendency to keratinize, they are more protoplasmic, and are easily distorted by local pressure, while irregular nuclear figures are occasionally found. Similar changes may be observed in chronic fistulæ opening on epithelial surfaces. The epithelial cells surrounding the areas of chronic inflammation are stimulated to activity and tend to reproduce their structures as nearly as possible. Other examples of this reaction to inflammation on the part of the epithelium, are found in lupus, ulcers from repeated burns, focal formalin dermatitis, *x*-ray burns, and varicose ulcers.

Each of these lesions has a common process, a reaction of epithelial cells to an inflammatory condition in a neighbouring tissue, but itself not an inflammation.

In viewing the causes contributing to the vegetative activity of the epithelial cells, several points stand out quite prominently. Tissues of all kinds are subservient to their vascular supply, and to the nutrient materials brought to them. There is a definite balance in which the functional activity of the cell and the energy supplied through the nutriment equalize each other. When this balance is arrived at, life in the cell may go on merrily, and without nervous control. With, however, the varying demands put upon a tissue, or upon the vascular source of supply, this balance is not easily maintained, and it is here that a nervous mechanism in reacting upon one or the other is required. Further, it is well to view the activity of the cell in the terms of energy available. Given that a certain cell has a definite amount of energy, we find that it may expend this in two ways. A certain amount of energy is required to carry on the metabolism of the particular cell, that it may functionate in its proper capacity in the general economy of the body. Another amount of energy is necessary to permit the cell to bring about a reproduction of its own kind, when such is necessary. We are familiar with the fact that the latter, the energy for vegetative activity, is very great in embryonic life, so that the cell has little or no energy left to carry on function; while, on the other hand, in adult life, the vegetative activity of the cells is almost at a standstill, and almost all the energy is devoted to the carrying out of the specific function of the cell. What forces direct the cell to expend its energy in one way or the other is not quite clear. Still, we have certain facts which indicate some of the causes which alter the activities of vital protoplasm. Broadly speaking, influences which alter the activities of cells, we call stimuli; and the important stimulus quickening the cells in processes of reproduction, is commonly some form of inflammation. Now it may not be that the reaction of inflammation in itself causes cells to multiply; but the combined forces, including the initial agent bringing on the inflammation, as well as the stimuli which develop during the course of the inflammatory process, each tend to irritate the surrounding cells to activity. These new stimuli consist mainly of chemical compounds, probably the products of cell disintegration.

The above conception is similar to that expressed by von Hansemann in anaplasia. Von Hansemann's hypothesis indicates that by some means certain cells have suffered modification, so that they no longer possess the differentiation exhibited by the mature tissue from which they were derived, but have acquired, or reverted to, a blastocytic type with increased powers of growth. In other words, von Hansemann gives us a statement as to the nature of cancer cells, but offers no explanation for the cause.

Ross has claimed that by using some of the decomposition products of cells he could increase the activity of the leucocytes, as well as that of the fixed tissue cells. He has observed the greater rapidity of growth of cells in chronic ulcers when these regions are powdered with "globin" or its products of decomposition.

In the closer study of the chemistry and structure of cells, it has been amply proved that the nucleus of the cell is the dominant structure, and that the initial activity for work is developed in this structure. It is through the vegetative activity of the nucleus that cells are caused to multiply. It may be even more, the chemical syntheses of cell metabolism, as Adami has pointed out, are probably entirely dominated by the nucleus. If this be true, then the condition which we commonly refer to as the selective action of the tissues, is a property of the nucleus. If the nucleus, by nature of its metabolic activity, has the property of being selective in its chemical reactions, and if it is on account of the adaptability of the nucleus that the tissues may accommodate themselves to varying conditions in the fluids which nourish the cell, then, too, disturbances which are effected within the cell structure will be first and most marked in the nuclear material. Our micro-chemical methods are quite inadequate to give any suggestion of the early action of deleterious influences which have the effect of altering the chemical architecture of the nucleus. It is only in the later stages, when a tissue has taken on, or at least has given evidence of, what we have come to recognize as true malignancy, that we observe an atypical structure in the nucleus. Von Hansemann has shown that the cells of human cancer are subject to a diminution in the number of chromatin filaments in the mitotic figures. Later, Farmer, Moore, and Walker observed an analogy between the mitoses of the reproductive cells and those of cancer cells. They believed that a fusion of an epithelial cell with another, possibly a leucocyte, would produce hybrid daughter cells with atypical neoplastic qualities. Bashford and Murray have also studied atypical nuclear divisions. They do not agree with Farmer, Moore, and Walker, but believe that the irregular numbers of chromosomes, sometimes more, at other times less, than the numbers of chromosomes in somatic cells, result from abnormalities in the splitting of the filaments or in the unequal attraction or faulty position of the centrosomes.

These observations indicate that when certain influences are present, or it may be are removed, the vegetative activity of the nucleus is exaggerated; and that one of the objective signs is the atypical arrangement of the chromosome filaments. It is a not infrequent observation to find one or more nuclear figures in gland cells in the neighbourhood of inflammatory reactions. The majority of the cells divide by typical karyokinesis. However, the atypical variety—at least atypical from the viewpoint of the size, shape, arrangement, and number of chromosomes—is also met with in inflammatory processes. Some of these examples of atypical "figures" are, we must admit, possible artefacts; there remain, however, some which have the semblance of true atypical mitoses. The point which is of direct interest is that we have in the reaction of tissues to certain irritants, alterations in the structure of cells which simulate closely those changes observed in malignancy.

Some observers have indicated that the direction of the mitoses of epithelial cells differs in malignant neoplasms from those occurring normally or in benign processes. Under normal conditions the growing cells standing upon a basement membrane are found to have the mitotic figures placed at right angles to the layer, while in malignant new growths this arrangement is not preserved. We have, however, repeatedly observed that the lack of regularity in the division of the nuclei is not confined to cancers, but may also be found in benign growths and in inflammatory states.

Of recent years we have had a closer study of known irritants which bring about atypical tissue proliferation. Workmen engaged in aniline industries have not infrequently kidney diseases and bladder irritation. The latter condition varies from a slight superficial inflammation to actual proliferative vegetations upon the bladder mucosa. A chronic cystitis precedes tumour development. Among those who have been engaged in this work over extended periods—ten years or more—the presence of papillary tumours is not uncommon, and the literature contains reports of some forty-five cases. The early stages of papillary tumours of the bladder are notoriously difficult to diagnose as to their malignant qualities, but of these semi-experimental cases in aniline workers, over three-fourths of the total number of tumours were observed to possess malignant features.

Equally important are the reports of papillary tumours of the bladder developing in those infected by the bilharzia. Egypt gives us the greatest number of these cases. The accumulated evidence of the association of bilharzia disease with new growths of the bladder is undeniable, and similar to the type of tumour in aniline workers, the malignant tendency is great. Goebel, after a careful and extensive study of bilharzia neoplasms, concluded that about fifty per cent. were malignant.

Ferguson has further collected forty cases of primary, malignant disease of the bladder associated with bilharziosis. He has had an excellent opportunity of studying all grades of bilharzia infection. In several cases of bilharzia of the bladder, the surface epithelium was increased in thickness, and columns or cylinders of the epithelium, continuous with the surface, extended into the connective tissues in the region of the inflammatory reaction. Various grades of this proliferative change were to be observed according to the severity and length of time of the infection. Ferguson states that he has "no hesitation in affirming that cancer of the urinary bladder is the irritation cancer of Egypt." These new growths produce metastases.

Recently Wolbach has discussed tumours in their association with repeated injuries. Among the many instances of the clinical relation of tumour to injuries received, he reports from his own experience the development of cancer of the skin to *x*-ray burns. These neoplasms, he says, constitute the first experimental cancers. The results of his studies showed that the chief effects of *x*-ray exposures were upon the tissues of the skin, connective and smooth muscle tissue, and endothelium. There is a repeated destruction and repair of the tissues following the exposure, and finally obliterative changes develop in the blood vessels which lead to nutritional disturbances. Wolbach believes that these nutritional changes are important factors in the subsequent development of cancer, and that the direct action of the rays does not lead to the tumour growth. There appears to be a "latent period" of several years between the time when the individual received *x*-ray treatment and the time of development of the new growth. The constant and severe changes existing over this period, in the deeper tissues, have a marked effect upon the epidermis, and are probably responsible for the acquisition of the new properties on the part of the epithelium. The progressive character of the skin lesions, from the earliest hyperemia to a definite cancerous growth, indicates the close relation of tumour to repeated injuries.

Quite striking were the results of B. Fischer, who was able to induce atypical epithelial proliferation by the subcutaneous inoculation of Scharlach R. dissolved in olive oil. The proliferative reaction of the overlying epithelium, sending large columns of cells into the underlying corium, was interpreted by Fischer as an attraction, or attractive force, on the part of the Scharlach R. for epithelial tissue. This special attractive force he called attraxin. The connective tissues react by a process of an acute aseptic inflammation. The study of the epithelial reaction is interesting. Mitotic figures are seen, cells of irregular size and shape are present, and epithelial pearls are not infrequent. Depending upon the depth to which the fluid is injected, one obtains an extension of the proliferation into the deep tissues. At times the new growth forms quite a mass, projecting above the surface like a diffuse wart. Metastases have never developed from these experimental proliferative masses, and the newly developed tissues always regress and tend to assume the normal as soon as the foreign materials inoculated have been absorbed and have disappeared from the local site.

Borrel reports the frequent occurrence of cancer of the skin in association with chronic lesions caused by the demodex folliculorum.

Thus the majority of observations on the association of inflammatory states with subsequent malignant growth, have been made upon skin and mucosal surfaces, but evidence is not lacking that an equally important association may be observed in glandular organs. We are familiar with the reactions in the glandular tissues of the liver in cirrhoses, and it has also been pointed out that the adenomata and primary cancers of the liver are associated with fibroses of this organ.

From the practical standpoint, the changes occurring in glandular and other tissues, as the result of chronic irritation, or it may be even of conditions having some of the ear-marks of inflammation, lead to the greatest difficulty in recognizing the beginnings of cancerous processes. No longer can we trust naked eye appearance, and much less does the size of the mass give us any clue as to the invasive characters of the aberrant tissues.

I have been particularly interested in the histological characters of nodular masses present in the breast tissue, and in the similarity in the appearance of some truly inflammatory reactions to those which have been demonstrated in metastases to have malignant qualities. There would appear, when one arranges a series of such specimens, to be a direct transition, in histological appearance at least, from the one with simple inflammation to the other having malignant qualities.

The reactions occurring in the breast tissue under the influence of irritants are to be observed both as to the changes in the stroma and to the effects upon the glandular epithelium. Particularly are those reactions which are of a chronic nature more important in the study of the comparative changes in inflammation and cancer, than those processes which are of a more acute nature. In the acute conditions, the reactions take place in the supporting connective tissues, where either the destruction of this tissue along with its contained glandular elements takes place, or, if the reaction is less severe, the affected areas are flooded by the inflammatory exudate. During these processes of acute reaction there is usually some attempt on the part of the fixed connective tissues and their functional gland structures to react, either in an increase of their normal secreting function or in some attempt to increase the tissue mass by proliferation. It is, however, more commonly found that in such acute inflammatory disturbances, where the causative agent is not sufficiently severe to lead to tissue destruction, the reaction in the glandular structures is commonly that of increased function or exfoliation of the lining cells. On the other hand, the reaction in the connective tissues is usually more pronounced, at least in its microscopic character, depending upon the intensity of the irritant. Those which interest us the most, have but little tissue destruction, but the tissues, besides showing the presence of exudates, also exhibit an increased activity in the fixed tissues themselves. This proliferation is mainly in the fibroblasts, and to some extent in the endothelial cells.

As we pass from the acute to the more chronic types of inflammation, the changes in the connective tissue are evidenced mainly in the increase of a firm fibrous tissue with a greater or less infiltration of lymphocytes surrounding the vessels and gland structures.

It is more particularly in this type of inflammation that our interests centre, as in it the changes become more evident in the epithelial tissue. Whereas little evidence of active proliferation is seen in the stroma, the tissue being almost entirely composed of adult connective tissue cells, there is not infrequently a great reaction present in the gland structures. The study of such inflammatory reactions includes the study of that large group of cases variously described as chronic interstitial mastitis, fibrosis of breast and fibrocystic disease of the mamma. In all of these there is the one common condition, that of fibrosis. In some cases the fibrous tissue is localized in nodules, in others it pervades the breast diffusely. The breast itself is harder than usual, and may feel nodular, or it may have a single firm mass directly beneath the nipple. At times, also, the condition follows the direction of the lymphatics, so that two or more nodular masses arrange themselves in a row from the region of the nipple in the direction of the axilla.

Our knowledge of inflammatory processes suggests in these cases a preceding acute inflammation, although the history of such is not always obtainable. It is to be observed that the inflammatory processes to be noted in the stroma are, in their spread, closely associated with the lymphatics surrounding the gland structures. Thus it is found, that in the periphery of the diffuse inflammatory reaction the outer borders show the main reaction about the gland structures. Occasionally, too, in chronic forms of the disease the main inflammatory process is to be observed in similar areas, and all gradations between the acute inflammation and fibrosis is present about the gland structures in different parts of the breast.

While some of the fibroid processes of the breast are isolated to nodular masses involving the glandular part of the organ, other cases show a diffuse process, in which the greater part of the breast tissue becomes indurated. Not uncommonly these changes develop at a time when the breast becomes more adipose and pendulous, so that the fibrosis is associated with the deposition of heavy layers of fat.

The point of importance, however, is that the fibroid changes take place about the glands and their ducts, and by direct mechanical interference still further impede the functional activity of the glands. Whereas the activity of these glands for the most part ceases with the menopause, their structure is well maintained for some time. A certain amount of secretory activity continues all through life—a physiological necessity—for all tissues must functionate to maintain a healthy equilibrium. Adequate nutriment is supplied to the tissues equal to the requirements of their work. When, however, by an external process the functional work of these cells is interfered with, they have a potential excess of available energy.

Not only does the fibroid change lead to stasis of the mammary secretion along with the development of cysts, but the very condition which has induced the process of fibrosis has, simultaneously, had an effect upon the character of the epithelial tissues. The gland structures are still further altered by the change of their environment, the metamorphosis of a vascular stroma to one of less activity and poor in its nourishment. The development of sclerosis of the vessels is a not infrequent feature which may have a bearing similar to that observed by Wolbach in the skin.

The association of chronic interstitial mastitis with cysts has previously been commented upon by Schimmelbusch, Koenig, Roloff, and others, so that we need hardly touch upon it here. It was noted that the condition was usually bilateral, and commonly extended diffusely through the breast tissue. The interstitial proliferative changes in the connective tissues are primary. The epithelium lining the cysts, which is the epithelium of the gland tissues of the breast, does not show a uniform character in all cases. In some it remains only passive—simply acting as a lining layer to the cyst, and becoming more stretched as the cyst becomes more widely distended. In other instances, however, reactive changes occur, so that instead of remaining as a single layer of epithelium, proliferative changes develop which entirely alter the histological picture as well as disturb the relation of the epithelium to its underlying supporting stroma.

It is of interest to study these epithelial reactions more closely. Of these we find two distinct types which can be recognized only by the microscope. On the one hand, there are cases in which the lining membrane not only clothes the dilated cavities, but in which papillomatous outgrowths develop with typical finger-like processes. These papillomata project into the cyst and consist of an overgrowth of the structures of the entire cyst wall. The stroma projects from the submucosa and ramifies into many processes, each being typically lined by a single layer of cubical cells. It would appear that the cyst wall itself has been stimulated to overgrowth. The epithelial cells are nourished by the vascular loops which ascend in the stroma of the papilloma. The development of these aberrant structures may be compared with the development of papillomata on other epithelial lined membranes.

Quite different, however, is the structure of the second form of epithelial overgrowth. In it we observe the proliferation of epithelial cells alone. There is no supporting stroma, and although large masses of epithelial cells gradually fill in the lumen, there are no nourishing blood vessels which accompany the growth of the epithelial cells. The growth of the cells is more or less concentric and inward, but the core of the epithelial cells may extend in either direction along the lumen of the tube. The activity of the growing cells may be observed in the rather frequent mitoses.

In this second form of epithelial growth, then, we are dealing with a proliferation of the lining cells, in which a proper relation of the epithelial cells to the stroma is no longer maintained. The structure is atypical and proliferation is active. Moreover, there is no longer a single growing layer standing upon a basement membrane, but proliferating cells are found everywhere in the new mass. These conditions, particularly, give us difficulty in determining the presence or absence of malignant qualities.

Schimmelbusch concluded that all forms of such epithelial overgrowth must be classified among the neoplasms. He regards them as developing sui generis, and applies the name adenoma mammæ. He, however, overlooks the fact that the condition is preceded by a low grade inflammatory reaction and cyst formation, and that all the cysts do not react in this manner. Moreover, as we have pointed out above, epithelial tissues do react to irritants, and these reactions vary according to the stimulus present. One other form of fibrosis of the breast must not be overlooked. I refer to the apparently spontaneous fibrosis which is so prone to involve the glandular elements and yet does not appear to be associated with an inflammatory reaction. I would not say, however, that an irritant or stimulus in some form was not present.

If, now, for a moment, we turn our attention to the growth of cells in general, we may offer some grounds for suggesting the close resemblance of these cell masses, stimulated to activity by irritants and inflammation, with those of malignant new growths. Our conception of the requirements of cells for their passive existence, as well as for growth, has been clouded by the teaching that a certain relationship must exist between the growing tissue and the blood supply for adequate nourishment. What an adequate nourishment consists of has never been defined. Quite recently, however, through the work of Harrison, Carrel, and Burrows, and more recently of Weil of our laboratory, it has been shown that the tissues of warm blooded animals, including mammalia, may be nourished and grown on diverse media. Plasma, serum, Ringer's solution, and Locke's solution, all serve as suitable media in which almost every tissue of the body may be grown. Connective tissue, heart muscle, liver, kidney, spleen, and skin have each been grown in our laboratory. The question, then, of tissue growth presents itself in a new light. We no longer throw the shroud of mystery around the processes of the so-called spontaneous growth. We may view it as a proved fact that our body cells live and multiply with great ease and that the quality of the nourishment is not special for each tissue, as was once held. This statement is, however, only true in the consideration of the needs for the vegetative activity of the cells. For special functions of tissues special food is no doubt required.

Applying these considerations to the proliferative processes of the breast, it is evident that the factors for the growth of the epithelial cells are present. The initial stimulus or influence acting upon the cells is received during the inflammatory reaction, which occurs in the interstitial tissue. With the development of the cyst cavities, a type of fluid accumulates which is an adequate nourishment for the growth of cells. What difference there is in the type of stimulus—the one which induces papillomatous overgrowth with stroma, and the other in which the epithelial cells alone react in proliferation—we cannot say.

From a general study of tissue reaction, it would appear that the character of the tissue growth is dependent upon the nature of the stimulus, the change in the nourishment, and the state of the cells acted upon. How one irritant differs from another in its effect is difficult to say—but it also must be conceded that the same stimulus, under different conditions, has widely different effects. Dr. Haythorn, of our laboratory, has been able to show that subacute and chronic inflammatory processes may at times lead to metaplasia of the bronchial mucosa, and that in this process the stimulus acts upon the growing cells, so altering their characters that a histological metaplasia results. However, the changes in metaplasia are not alone confined to histological characters. The entire nature of the cells may become modified so that the vegetative qualities are also altered. This we believe happens in the proliferating cysts which we have described. Moreover, the degree to which this metaplasia may be carried is very variable, so that all proliferating epithelial masses differ from each other quantitatively in the changes produced. I use the word metaplasia in the broader sense, and do not confine its meaning or interpretation to that as defined by Virchow. In metaplasia I see not only a change in the form and appearance of a cell, but also consider changes in the internal architecture of the nucleus and cystoplasm, as well as an alteration in the physiological function of the cells. These latter aberrations may be great or small—and in fact cannot be accurately determined by present methods. Nevertheless, these slight metamorphoses have an important bearing upon cell relationship in the tissues where they occur.

It is obvious that all living matter is influenced by its environment. Our body cells are just as impressionable masses, to external influences, as are the more lowly organisms, particularly bacteria. New qualities may be acquired, and where once accepted are firmly impressed.

In the study of seventy-five cases of breast conditions of various kinds, I have been able to observe transitional changes from the simple, smooth lined cysts to those in which the epithelial proliferation indicated malignancy with metastasis. Although the distinct types of these processes which stand at either end of the series are clear cut and offer no difficulty as to the extent of their variations from the normal, I have found processes in which a conclusive diagnosis was impossible. Some of the instances of epithelial metaplasia, which may not at the time of examination indicate the crucial qualities of malignancy in metastasis, nevertheless possess features which we have learned to respect and to fear, lest in the future they should acquire this character. It may be that the forces acting upon the epithelial cells have carried the process of metaplasia to only a certain degree, and that these partly altered cells remain in a latent or semi-functional state until the advent of other new stimuli which bring about their complete metamorphosis. Latency of tissue in this sense is to be distinguished from the latency of the vitality or life of cells as has been discussed by Carrel.

Up to the present no one has been able to observe the beginning of a cancer. We have, however, had an opportunity of studying an unusually early cancer of the breast. The entire mass, which was only discovered by accident, was only half a centimetre in diameter, or about the size of a pea. Microscopically all the characters of malignancy were present, but there was no clinical verification of this diagnosis. However, this small mass showed evidence of destructive infiltration, the development of islands of proliferating epithelial cells with both typical and atypical mitoses. In a few places the proliferative changes did not differ from the type of epithelial proliferation which is found in cysts in which the stroma does not react. These latter areas, moreover, did not show evidence of a destructive invasion, and the basement membrane remained intact. It did appear that these areas were in the stage of transition in which the epithelial cells had acquired some new qualities, but were not completely metamorphosed. Moreover, many of the gland structures in this cancer were in a process of metaplasia—if we may so call it—in which several portions of normal glands were simultaneously taking on new characters. In other words, it did not appear that the growth had arisen from a single cell, but from a mass of cells which had been similarly influenced. Moreover, the incomplete changes which had occurred in the outlying parts of the growth did not differ in their appearance from epithelial changes observed in benign cysts of other breasts.

In this conception, then, the Cohnheim theory of embryonal cell rests is unnecessary in explaining some breast cancers. Further, a metaplasia of epithelial tissue, giving it malignant quantities, may occur *in situ* in gland structures without having a mechanical or vegetative displacement as suggested by Ribbert.

Cysts of the breast with reactive proliferative changes in the epithelial lining stand in an intermediate position between true inflammatory processes and cancer. Those cysts in which the epithelium alone shows vegetative activities have many features in common with true neoplasms, and must be viewed with grave suspicion.

Evidence, therefore, indicating a relationship between inflammation or irritative processes of tissues, to cancerous conditions, are not wanting either from clinical or microscopical sources. As we have then come to accept these observations and interpretations as facts, we must also be willing to appreciate the difficulties which must arise in those borderland conditions which, having obvious inflammatory or reactive changes, also have some of the characters of early malignancy.

At the present time in the practical crusade against cancer by the surgeon, the early diagnosis of the process is most important. Radical operation is still the important measure of success. It has come, therefore, that the former stereotyped points for clinical diagnosis of cancer are almost valueless in coming to a conclusion whether a tissue has benign or malignant qualities. The most satisfactory evidence, although not always conclusive, is gained by the examination of the tissue after removal, both by macroscopic and microscopic means. Nevertheless this technical procedure must be preceded by the collection of all possible clinical data. The differential diagnosis of any given case must rest in the hands of the clinician, who obtains additional information upon the case from all the allied departments of medicine. In the hope of early diagnosis, every means must be sought to give assistance in the better understanding of the disease process, for we have learned that our hopes in cancer are in the early removal, before the malignant character of the growth has become dominant in diverse parts of the body.

The surgeon has for years been insisting upon the sending of cases with malignant neoplasms to him early, and although much has been done in this direction, we have to-day reached a point where it is quite impossible to make an early diagnosis by clinical means alone. Small tumorous masses are often observed by the practitioner, but in this early stage even the keenest observer would hesitate to give a conclusive answer to the question of malignancy. Fischer-Defoy points out that out present attitude, gained by the experience of early operation on doubtful cases, is one of intense alertness, weighing in the impartial balance points gleaned in each case. The cases are frequently observed so early that cachexia, anæmia, and wasting may all be absent. Fever, ulceration, and chronic discharging sinuses, are only observed in the neglected conditions.

Thus, for the pathologist, too, the diagnosis of cancer has undergone much change. Not only has a new laboratory technique replaced the old, but the routine tumour tissue received by the laboratory is of quite a different character. The laboratory is approached by the clinician for the purpose of establishing a diagnosis, and not to verify the diagnosis. The masses of doubtful tissue are often small, the entire tumour not being over one or one and a half centimeters in diameter. With such small tissue masses, the microscopical examination of which is to determine the malignancy and prognosis, the microscopist is assuming great responsibilities, of which he is not forgetful.

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RHEUMATIC FEVER AND THE ARTERIES

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Cardiac lesions are no longer considered as complications of acute rheumatic fever, but are just as much a part of the disease as the affection of the joints. The frequency of the association of pericarditis, endocarditis, and myocarditis or any one of them with other lesions making up the picture of acute rheumatism is very great, and I think many will agree with Aschoff that every attack of rheumatic fever leaves its mark on the heart. It has been particularly emphasized that the myocardium is most often affected, and this for the reason, as we shall subsequently indicate, that the reactions to rheumatism occur in the neighborhood of the fine nutrient vessels of the myocardium. Inflammatory exudates occur about these fine arteries, and are replaced in the chronic stages of the disease, by fibrosis.

In general our conception of acute rheumatic fever is being modified with the progress of the pathological studies made upon it. That it belongs to the acute infections no one will deny, and many are willing to place a considerable stress upon the organism of Poynton and Paine as the causative agent. Whether it is the initial factor, or whether some other condition predisposes to the invasion of the diplococcus rheumaticus, we cannot here argue; but it is more than probable that the lesions as we find them in diverse parts of the body are the result of the action of these diplococci.

In the late stages of the disease process we not infrequently observe the association of heart, kidney, and arterial lesions making up a picture very different from that of acute rheumatic fever.

Nevertheless we are compelled to admit that all of them are either the result of the primary acute process or else that in the process of a vicious circle one lesion has followed the other. A close study of the various organs can alone determine the direct effect of this systemic disease upon each, and with this in view I have followed, in a few cases, the lesions to be observed in the arteries and more particularly the aorta during the course and different stages of rheumatism. This study presents purely the pathological side of the question. The cases studied were selected from a series of autopsies, only those being taken in which one or more previous attacks of acute rheumatic fever were established in the clinical history and in the presence of interstitial myocarditis.

In classifying the cases here examined I have divided them into three groups: (1) Acute rheumatic fever, first attack; (2) recurrent rheumatic fever, and (3) chronic rheumatism. It was interesting to find that the changes in certain arteries were analogous to the lesions found in the heart in the different stages of the disease; in fact, the resemblance of the inflammatory reaction in the aorta to that of the myocardium was very great. It may, however, at the very beginning, be pointed out that the causative agent of acute rheumatic fever has a predilection for the perivascular tissues of the smaller vessels, the arterioles, and does not appear to disturb the larger arteries directly, save by damage in the vicinity of the vasa vasorum. Up to the present I have not made a systematic study of the arterioles of all the viscera of the body. My observations have, however, indicated that the arterioles of the internal organs, except the circulatory system, are not attacked with any constancy.

The aorta shows a fairly typical lesion. This lesion is particularly to be observed in the ascending limb and in the arch, less frequently in the descending thoracic and seldom in the abdominal aorta. The distribution of the process in the aorta is similar to that of syphilis, and, as a matter of fact, to that of many bacterial infections. The main coronary arteries of the heart are inconstantly involved, while, on the other hand, the finer ramifications of this vessel are invariably affected, being a part of the myocardial disease which forms an important factor in the pathological picture of rheumatic fever. Moreover, the arterioles passing to the serous cavities, particularly the pericardium and the joints, partake of a reaction similar to that of the nutrient arteries of the aorta and heart. In how far the vessels of the meninges and the abdominal viscera respond to the irritant present in rheumatism I am not prepared to say.

ACUTE RHEUMATIC FEVER (FIRST ATTACK). Of such cases I have had only three to autopsy, aged nine, sixteen, and nineteen years respectively. All of them had associated cardiac disease, two with pericarditis, all with acute interstitial myocarditis, two with fresh mitral deposits, and one with recent mitral and aortic vegetations. The youngest case had suffered choreiform attacks during his illness.

The arterial findings being similar in these cases a single description will suffice. Macroscopically the arteries were but little changed, the oldest individual alone showing superficial fatty streaks in the intima. The elasticity of the aorta was not altered and none showed the inflammatory foci to the naked eye. Microscopically, however, there was a very striking picture present in each case. Sections of the ascending and transverse portions of the aorta showed that the intima was not thickened and the lamellæ of the media appeared regularly disposed. In the media, however, the arterioles appeared more prominent than usual, and these nutrient vessels encroached beyond the outer third into the muscularis. These arterioles had an edematous perivascular infiltration in which aggregations of lymphocytes and plasma cells were constantly found. Moreover, the elastic fibers of the media in this vicinity were interrupted, often appearing as if mechanically broken. Muscle elements of the media in the neighborhood of the vasa vasorum had also, in part, disappeared, an indication of some local toxic agent surrounding the nutrient vessels.

In the adventitia the infiltration by inflammatory cells was very extensive. Lymphocytes and plasma cells were diffusely scattered through the tissue, while more densely aggregated cells were seen about some of the vessels. Polymorphonuclear leukocytes were also seen, but in relatively small numbers. Occasionally areas indicating endothelial proliferation were observed in the vicinity of the capillaries. The connective tissue was loose and rather edematous, while the capillaries were much congested. In some of the reactions fibroblasts were apparent, but no new connective-tissue fibers were observed.

The main coronary arteries of the heart were but little affected, save at the periphery of the adventitia. The smaller arterioles, however, showed a dense infiltration about them, with a considerable change in the appearance of the heart tissue in the immediate vicinity. In places the infiltrating cells were mainly lymphocytes, while in others again leukocytes and endothelial cells appeared to make up the main type of invading cell. The heart muscle adjoining these involved vessels showed evidence of degeneration and not infrequently complete death. In the place of these heart muscle cells there remained a homogeneous and almost structureless material with occasional evidence of replacement by fibroblasts. The perivascular infiltration was very widespread and quite uniform throughout the heart muscle.

It is indicated in the above that the main reaction occurring about the arteries during the acute stage of rheumatic fever is in the outer coats. The adventitia and the media are probably simultaneously involved, at times by way of the nutrient vasa vasorum, or in the case of the smaller vessels, the arterioles, directly from the perivascular lymph stream. The reaction is of the acute non-suppurative variety, in which the lymphoid type of cell and the endothelial cells play the important part. Similar to the reaction in the heart, the tissues of the arteries suffer directly from the harmful agent of acute rheumatism, in which both muscle cells and elastic fibers in the immediate neighborhood are destroyed.

RECURRENT RHEUMATIC FEVER. Four cases of recurrent rheumatic fever were studied. Their ages were twenty-eight, thirty, thirty-six, and forty-four years respectively. In each case there was a history of one or more previous attacks, and the heart showed the presence of subacute and chronic interstitial myocarditis to a varying degree. Moreover, some form of recurrent endocarditis was present in each heart.

Sections of the aorta showed the intima to be fairly intact and with no definite areas of thickening. The arrangement of the media appeared quite normal and regular in its inner half. However, it was observed that the vasa vasorum were quite frequent in the middle third of the media, and that these small bloodvessels were surrounded by an excessive amount of fibrous tissue, along with a cellular infiltration consisting of lymphocytes, plasma cells, and a few endothelial cells. This perivascular infiltration was quite constant, and at times the aggregation of wandering cells was quite marked. In some areas it was definitely indicated that some of the muscle cells and elastic fibers had been destroyed and their place taken by a meshwork of new connective tissue. Polymorphonuclear leukocytes were for the most part wanting.

The adventitia also showed a lymphocytic infiltration around the vasa, as well as an increase in the connective tissue close to the media. Some of the nutrient vessels showed a thickening of their walls with a narrowing of their lumina.

It is evident that in these cases of recurrent rheumatic fever certain changes have been produced about the aorta, which subsequently lead to an increase of fibrous tissue in the outer portion of the media and in the adventitia. Moreover, it would appear that with each recurrent attack of the disease a new non-suppurative inflammatory reaction occurs in the sites of the former lesion. Therefore in the recurrent types of the disease various stages of the inflammatory process may be observed in the same specimen. The former attacks are indicated in the presence of adult connective tissue, while the recent progressive condition is seen in the non-suppurative inflammatory infiltration.

Chronic Rheumatism. All of the eight cases considered in this class were such as had suffered, for a considerable time, recurrent or progressive heart disease associated with acute rheumatic fever. The majority had died of broken cardiac compensation. The most constant cardiac finding was a chronic interstitial myocarditis, while an old endocarditis involving one or other of the valves was not uncommon. In this series of cases the lesions of the aorta and of the smaller coronary arteries was quite constant, but varied in the extent of the reaction. Macroscopically the intima was more or less nodular. At times the thickening of the intima was quite extensive, while, however, it showed little evidence of degeneration or atheromatous change. There was usually a great loss in the elasticity of the entire vessel wall, and the artery felt thicker and heavier.

Sections of the aorta showed that the thickened intima consisted mainly of a hyaline looking fibrous tissue, in which numerous fine elastic fibers were seen. The media appeared compact and dense, and the muscle cells did not appear as frequent as usual. The vasa vasorum were seen commonly in the middle third of the media, and some were found in the inner third. The majority of these vessels had their direction parallel to the elastic fibers and a considerable number were surrounded by a fibrous tissue, which had replaced the muscle cells and interrupted the concentric layers of elastic fibers. A slight lymphocytic infiltration surrounded many of them, and plasma cells were also present. Polymorphonuclear leukocytes were not present. In the adventitia there was a fairly dense stroma of connective tissue built closely upon the outer border of the media, so that the thickness of the entire wall was increased. The small bloodvessels were not congested, but in their vicinity there were some scattered lymphocytes. The vasa vasorum in the adventitia had thickened walls.

The changes observed in the vicinity of the coronary arteries of the heart are those with which we are all familiar in chronic interstitial myocarditis. In fact, these very interstitial myocardial changes which have attracted our attention are the result of non-suppurative processes occurring in the neighborhood of the finer coronary arteries. Thus the pathological process about the arteries in the late stages of rheumatic fever is one of inflammatory fibrosis. This fibrosis is particularly evident in the first part of the aorta and about the smaller branches of the coronary artery of the heart.

Discussion. There appears to be a fairly definite form of arterial disease which is associated with rheumatic fever in its different stages. The arteries react to the irritant in a true inflammation and this reaction is to be observed in the adventitia and the outer portion of the media. In the acute stages the inflammatory exudate is of the non-suppurative variety in which the lymphoid cell is most prominent. The inflammation occurs particularly in the neighborhood of the smaller arteries, while the medium-sized vessels are little affected. On the other hand, the larger arteries, which are supplied in their outer coats by nutrient vessels, are damaged by the inflammatory process which travels along the vasa vasorum.

During the acute process the fixed tissues in the immediate neighborhood of the small arterioles are damaged or even destroyed, but abscesses are not formed. In the aorta the destruction of tissue is particularly noted in the loss of muscle and elastic elements. Similar degenerations are common in the heart. At times the degenerative processes are quite extensive, leading to the loss of considerable tissue of the part, and probably weakening the media. The intima of the arteries does not appear to be primarily affected. In the early stages a slight superficial fatty change is sometimes noted, while later, in the chronic stages, the intima proliferates, giving rise to a nodular endarteritis.

The repair, following the inflammatory process of the media and adventitia of the arteries, is by fibrous tissue. Thus the aorta in chronic rheumatism is disturbed by a patchy fibrosis, better spoken of as chronic productive mesaortitis. It is evident that the arteries which are involved in a chronic mesarteritis have lost much of their elasticity and may become subject to a diffuse dilatation of their lumen.

In some respects the acute stage of the mesarteritis resembles the early process of syphilis, but with this difference, that the destruction of the tissues in syphilitic mesarteritis has other qualities, in that the lymphoid infiltration about the vasa vasorum is greater, gummy necroses are often seen, and granulomatous tissue surrounds the areas of greatest reaction. The invasion of the capillaries through the media toward and into the intima is more marked in syphilis, while the nodular endarteritis is also far more decided.

In 1903 Chiari suggested the division of productive mesaortitis (as well as mesarteritis) into two groups, type "A" and type "B" respectively. His type "A" is a chronic inflammatory disease of the media secondary to an intimal process. The mesarteritis type "B" is a primary inflammatory process involving the media, which is usually of infectious origin and most commonly due to syphilis.

As I have on a previous occasion pointed out, Chiari's classification is a good one, but it must always be borne in mind that this type "B" (productive mesaortitis) must include other infectious inflammatory conditions of the media than syphilis. As has been indicated above, the lesions occurring in the arteries in rheumatic fever belong to the group of productive mesarteritis type "B."

The Medical Profession, Public Opinion and Animal Experimentation.

By OSKAR KLOTZ, M.D.,
Pittsburgh.

(Read at the General Meeting of the Medical Society of the State of Pennsylvania, Scranton Session, September 25, 1912.)

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he Medical Profession, Public Opinion and Animal Experimentation.*

By OSKAR KLOTZ, M.D.,
Pittsburgh.

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n answer to the invitation of the state ical society to discuss this important c, I have been urged to accept by one sideration. Briefly, this is for the full- nderstanding and exposition of the tion of the medical profession to the lic, as well as the present-day attitude he physician and laboratory worker to lic opinion upon questions mainly ical.

; is not an infrequent occurrence that y opinions are offered by each and y one upon questions which are foreign his individual profession. To a cer- extent our aptitude to form these y opinions is dependent upon our close ciation with the not unbiased opinion he daily papers. Politically, without tation we rely for our information nly upon the daily press and periodi- We have little time, even if we were nclined, to enter into a serious research btain the truth upon each clause in the form of a political party. And yet e are matters of our daily life. We interested in politics because of their association to our own interests and home. In such matters it would ap- that every one, who remained even nly a slight extent in touch with his y surroundings, should be fairly well pped to discuss the politics of the gov- nent and the value of long delibera- s by our political representatives. It is not, however, until we have placed ourselves in the position of one who gives initiative for new acts or one who must for his livelihood closely study a particular trade or profession, that we appreciate even in the smallest way the intricate problems which confront that particular work.

It is probably true that in none of the sciences does the individual student or critic require to be armed with such diverse knowledge and experience to have a proper understanding of his subject as in medicine. There is a long history indicating the evolution of medicine and its allied sciences. Much of what has occurred in the past influences us but little in our present position, but nevertheless the art and science of medicine has accepted much from the older teachings, particularly in placing the profession above the quality of a trade and by imbuing each of its associates with the seriousness of the work undertaken and the necessity of truthful observations for advance.

It is impossible for anyone to indicate the quality of the mind which has led the profession on the up-hill climb to the position which it occupies to-day. The character of medical thought and persuasion is the composite aggregation of experiences gathered by generations from many fields.

It has for example been through long years of observation and research that certain social laws for the prevention of smallpox have been established. These observa-

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rom the Pathological Laboratories, University of burgh.

tions were not made haphazard nor were they evolved upon antecedent theories, for the theory only followed the actual observation. At present it is not our point to discuss the value of vaccination, yet there are individuals who, without experience, either in subjects of immunity, health or disease, attack the findings of students in this work. We can not too strongly denounce such an attitude. We would offer the same criticism to each one in the professional world who, inexperienced, deemed it his privilege to offer the last word in a foreign field. For the last fifty years the medical profession has been unusually active in determining causes of disease and indicating the prevention thereof. Much of the knowledge which has been acquired through hard work is looked upon by some as the natural course of events which would have become the property of the public even though the medical profession had not existed. Of course a discussion, with such opinions, is futile and leads to no end. I would even say that many of us in our own profession do not realize and impose sufficient weight upon the difficulties which had to be overcome to reach the status of the profession to-day. The martyrs in medicine are legion, but it would be ungrateful for me to offer their names in a discussion which to some still remained unconvincing.

What I wish to emphasize to-day is that we in the medical profession must come to a closer understanding with the public. In years gone by there was a time when the physicians and surgeons were a class who stood quite apart from all other people. In the mysteries of ignorance which surrounded medicine at that time, and it is not so long ago, there was reason for maintaining a certain secrecy upon individual opinion and methods of treatment. However, as new light was shed upon the dark places the public became interested and themselves enthused in the progress made by their students. Not only did th
come interested in what they realize
for their own happiness and welfar
they encouraged the work by offering
eys, buildings and public support to
on investigations in untrodden fiel
must be patent to all that, as indivi
little could have been accomplished
out the hearty support of the
Hence it is, that we as a professio
under an obligation to our fellows
though not one of us, share our fa
and successes with us. The closest
tion between the professional work a
public is best indicated in the inter
hibited in the municipal, state and f
hygiene departments.

It thus appears to me that we mu
be hasty in condemning the publi
wishing to know more intimately som
of the inner workings of the medic
ences, and instead of allowing only
of our work to filter through to the
there should be a careful exposition
findings portrayed in such language
may be understood by all. The b
medicine is written in words of tw
lables.

On the other hand the public
advised to attempt democratic rule an
ulation of matters which are of vit
portance for the progress of the m
sciences and for the betterment o
health of the public. We may onl
dicate that it is but a short time sin
public has taken an interest in sci
medicine and has given it proper su
It needs no long research in the hist
medicine to find that those who by
works have placed the milestones i
progress of scientific work, were aide
little or not at all or were even ham
by the attitude of the public opinion
work of Paré, Vesalius, Harvey, H
Jenner and a host of others, received a
ance from no one outside of the profe
and it was only after each of the

nstrated the truth of his researches
general sentiment acknowledged their
c work. However, as the value of
researches became known and as the
er of men who were willing to sacri-
their time in these researches in-
ed, then did the people recognize and
er medical investigation. It is quite
er that this should be as it was; name-
hat the benefits of the individual re-
hes should be definitely proved before
ther demand was made for increased
ties for work. But the brief period
h has elapsed in which the untrained
has permitted itself to become
iar with research, some demanding a
me of study, has been insufficient to
a proper appreciation of this work
he public should not demand so much
hamper in any way the progress of
tigations already begun. Unfor-
ely such has been the attitude of
who, I fear, fall into the category of
'uninformed.''

would seem superfluous to discuss the
of certain animal experiments that
arried on for the purpose of throwing
light upon old diseases, or that are
for the purpose of making a diagnosis
dividual cases. This subject is so
vorn that I fear much that I will
to say is a mere reiteration of a story
has ofttimes been repeated. Particu-
is this true as the medical profession
of recent years, had seriously to dis-
and to advance arguments to illus-
their sincerity in the value of these
iments and in the benefits which they
r upon humanity at large.

o often has this subject been discussed
r the term ''vivisection.'' The very
cter of the word has led to many mis-
ptions, so that he who is smooth of
e can paint word pictures which will
e the innocent. There are probably
with us to-day who have not shuddered
ragraphs of description illustrating the torture of animals in days gone by, or of semireligious festivals at which animal sacrifices were offered. Even the human was in fear of the application of these cruel methods, which in ignorance and superstition were applied to one to save or soothe the ills of a more influential individual.

We are, however, to-day extremely proud that mankind, particularly through the efforts of the medical profession, has advanced beyond the stage of superstition and has a mind of the inquisitive type, wishing to know the why and wherefore of every process in nature. Instead of assuming that frame of mind, fearing to trespass on holy ground, fearing to meddle with nature's works, and even be denounced when such was done, we are willing to-day to sacrifice those conscious comforts where ''ignorance is bliss,'' to labor in untrodden fields and to uncover many of nature's mysteries. We repeat, that we are proud of the attitude assumed in medicine to-day, that she is assisting to lift the veil, particularly to disclose the dangers which have confronted human life as well as the welfare of all animals.

To-day man is the keeper of everything living; upon him depends what formerly was discussed under the survival of the fittest. Though in the wild and untamed world the survival of the fittest is dependent upon brute force as well as keenness of intrigue, to-day man in the civilized portions of the globe must bear the onus of responsibility for the preservation of life. Things are changing and many of these changes are the result of the work of man. Just as with the advance of civilization, and the development of the biological sciences there has been an enormous growth in the population of all countries, so must the very face of the world change. But with this advance man must encounter, continuously, new features which are to govern his future as well as that of everything living. It is with the knowledge of this progress in the past that every means is utilized to improve the conditions for the survival of the fittest in the future.

The advances which we to-day claim are not alone in one science, but enormous strides are being made in every science, industry and art. The eagerness for the advance of each is so great that no one science can afford to lag behind another. Scientific advances are made only by receiving assistance from ancillary subjects, and so medicine can advance only with a thorough knowledge of biology, chemistry, physics and the allied departments.

More than two thousand years ago the monarchs of India and China, fearing the plotting of their subjects, dreaded death by poisoning. Particular individuals, therefore were charged to test the foodstuffs by feeding them to animals. These were probably some of the earliest procedures in which animals were used for the determination of a certain fact. For the kings it was a legitimate procedure to which they felt nobody could object. Some centuries later more pointed experiments were undertaken to determine the character of certain vegetable poisons as well as to obtain some knowledge as to the strength with which they could be administered. However, in those ancient times too great a relation between the action of poisonous drugs and the temper of the gods was given, so that the experiments were not disclosed in the proper light. The idiosyncrasy to the action of drugs was a process of fate or due to an external influence of demons rather than to altered inherent qualities of the individual. Thus being persuaded by hallowed myths, the experimenters themselves were unable to draw proper conclusions.

And so time passed on through centuries from Hippocrates, to Galen, Avicenna and Celsus down to the seventeenth century,
when a new light began to dawn in t
tory of medicine. Actual observati
all matters of science were then re
and the sequence of the processes
natural phenomena were appreciat
could be but a short time from the
ning of such truthful observations th
value and relation of each fact to th
eral principles of the science were give
proper weight. Every science, like a
passes through three stages in the h
of its development. In the first sta
servations are made very much at r
and without any thought of their va
consequences. This in medicine is t
pirical stage. Later on these fac
brought together in an orderly mann
the general findings are compared
uncommonly it will be found that t
sults of these observations indicate
mon principle governing the process
which the observations have been
Then we enter upon the second
Now in the ordinary course of even
first and second stages are deeme
many as being quite sufficient to est
laws, and though this is true, in so
these observers are willing to go, th
secutive observations upon phen
of disease, in the natural
of events, are difficult to
are impossible to control, and
seen factors can not be properly g
It is quite impossible to state how
this or that factor, in a given proces
played in bringing about a given
Therefore, it is necessary to have
means by which we can weigh each p
a reaction and each part of a phenon
There is but one way to carry this o
that is to put each factor to test.

Science has waited a long time f
third stage, the stage of experiment
In this, each observation which has
carefully recorded in the first stage a
been assembled in the second stage
to test and the weight of each ingr

ven its proper scale. No longer does
observer depend upon impressions
h he has received in his natural ob-
tions, but each idea must be replaced
ct.

ese three stages of growth are plainly
nt in the science of medicine. Un-
nately, however, the first two stages
ntermingled with so much mysticism,
we are unable to place much reliance
any of the observations. Unanswer-
phenomena lead to much speculation
h with the imaginative mind leads us
stray from the truth of the phenom-
nd the observation.

to the middle of the nineteenth cen-
there reigned the greatest empiricism.
ng the latter part of this period much
vay was made to classify observations
o indicate facts. Close relations be-
h different processes were recognized
interpretation of these observations
attempted. With the eagerness of
ting truthful observations there also
h the antecedent to experiment,—re-
on. With due reflection facts become
lated into hypotheses and the truth
pothesis rests in experimentation.

tle do we realize the advances that
been made since the hypotheses have
put to test. Questions in the biologic
ces can only be tested in their natural
on in the living body. It is only
sixty years since investigators have
eciated that to place their hypotheses
beyond the reach of destructive criti-
each factor associated with the phe-
non under discussion must be tested
The test of the living cell can not be
by mixing inert fluids and we are
ght face to face with the problem of
naterials in which questions of hu-
life are to be tested.

o often is the opinion expressed that
ractical physician and the laboratory
occupy two distant fields. This should
not be true. In fact, we welcome and admire those who are able to combine the two under the same roof. The one is but the assistant of the other and upon the second does the former place his hopes for his better work amidst humanity. Want of time is the greatest factor tending to divorce these mutual interests.

The reiteration of the benefits which mankind has obtained since the discoveries of Jenner a hundred years ago, is almost unnecessary. To recount the many applications of animal experimentation which has placed in your hand an instrument by which you are able to cope with disease would occupy a long time. Many in carrying out their missions of dealing with the sick are forgetful for the moment of the struggles by laboratory workers which made it possible for them to make a diagnosis and to carry out a successful treatment. Many of these discoveries could only have been made by the use of animal experimentation to prove the final point in the determination of the etiologic factor of disease. The character of these men who have worked and slaved in the laboratories, who have attempted, sometimes successfully, at other times without avail, to prove the *causa morbi*, can be read in the tragic histories of Reed, Lazear, Dutton, Ricketts, and others, each of whom offered his life, a sacrifice to science.

With the information that is now at hand we wonder that more did not perish in the early days of bacteriology. It is probable that over three quarters of the cases of ill health and disease which are treated by the internists are either the direct or indirect result of bacterial invasion. We well know the scorn with which Pasteur's inferences of the bacterial nature of certain diseases were received in the early sixties. We to-day would not have even this information had he not been successful, particularly with the later assistance of Koch, in reproducing the same diseases by the inoculation of pure cultures of bacteria.

The work of Ehrlich, Yersin, Kitasato, and Roux is too frequently neglected when in our daily work we are treating patients with antitoxin and other sera. The value of these need not be discussed, for if we gauge the importance of their discoveries in the terms of diphtheritic antitoxin alone, it is impossible to estimate the value in human life. There are, however, examples which are better gauged in money value. The causative agent of anthrax, black leg, swine plague, glanders, and actinomycosis was for centuries in question, and only after the proof by animal experimentation of the direct relationship of certain microörganisms to these diseases was established, has the government instituted hygienic measures, which in this country alone saves us millions of dollars annually. And all this information was received only by proof in animal experiments.

The relation of the pneumococcus to pneumonia, the meningococcus to meningitis, the *Spirochaeta pallida* to syphilis, and many other organisms to definite forms of human ills, have been and can only be determined by reproducing the
as demanded by Koch's third post

In particular has bacteriology
fertile field in which the value of
experimentation is very paten
pathology proper, new fields have
been demonstrated in which the co
tive study of human diseases in
mentally produced lesions has b
greatest value. The etiological fac
nephritis have always been a so
polemic controversy since the da
Bright. These to-day are disclosin
nature and it is probable that in
short time all will be as an open
Studies in edema and heart diseas
likewise been vexed fields of disc
Here, too, we can indicate that the
edge which we do possess has be
tained by means of studying fact
fact, factor upon factor, and obtaini
actual observations where we can
them, in living tissue.

I hold no brief for the carrying
promiscuous animal experimentat
any science. It is evident, howeve
progress can only be obtained by th
onstration of truth and the truth of
tissue lies in those tissues themselv

PUBLICATIONS FROM THE
University of Pittsburgh Medical School
PATHOLOGICAL LABORATORIES

TONSILLITIS

A HISTO-PATHOLOGICAL STUDY

BY

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SEPTEMBER, 1912

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TONSILLITIS.

A HISTO-PATHOLOGICAL STUDY.

By W. W. G. MACLACHLAN.

Pathological studies of the faucial tonsil are quite numerous in the literature, and consequently there are very few points pertaining to the pathological conditions which have not been brought forward and discussed at considerable length. However we are still a long way off from having the last word said upon the various diseases affecting or associated with the tonsil. As a rule the discussions upon each of the different pathological conditions affecting the tonsil have appeared in separate monographs. Tuberculosis of the tonsil has been an especially favored subject of many articles. The clinicians have been very prone to throw much blame upon the tonsil, for its association with systematic diseases and infections, while little or no positive evidence is offered for this view. Our ignorance of the function of the tonsil has allowed much latitude for speculation and the reiteration in text-books of much presumptive evidence has led to dogma. Hence with all the laboratory work which has appeared there is no unanimity of opinion upon any of the important problems concerning this organ.

In this paper we have incorporated discussions upon many of the pathological changes observed in the tonsil, while with some of them (inflammation) the discussion has been enlarged upon more fully.

Through the kindness of Dr. B. M. Dickinson, I have been able to examine over three hundred and fifty pairs of tonsils. The materials were collected by him from the nose and throat clinic of the Mercy Hospital at Pittsburgh.

The routine proceedure of examination was similar to that carried out for other surgical material. In the majority of cases the tonsils were put into fixing fluid immediately on removal. Zenker's fluid and formalin as fixatives were first used, but later only Zenker's was employed. The sections were cut in paraffin and stained with eosin and methyloene blue or haematoxylin and eosin. Special stains were also used for cell study, stroma and for bacteria.

The materials were sent to the laboratory as routine surgical specimens for diagnosis. It was soon found that the ordinary routine methods for diagnosis, which are commonly carried out on other tissues, were quite inadequate. Particularly was it found, that a correct interpretation, of the so-called "hypertrophied tonsils" could not be obtained by examining only a few sections. Hence we set ourselves the task of studying each case, of cutting sections from different parts of each organ and staining these by a variety of methods. Thus my work was rapidly increased but I believe the time and labor were well repaid in the better results obtained. It was not infrequently observed that different crypts of the same organ showed lesions of different characters, or that by obtaining a series of sections, different lesions would be found in the same crypt. For example the rather frequent ulcer of the tonsil is only accidentally encountered in routine examination, while it is commonly observed when properly looked for. In this manner, the work which was at first undertaken, has been enlarged to consider the various inflammatory processes of the tonsil, with their sequelae.

FUNCTION OF THE TONSIL.

Before entering into the discussion of the pathological conditions of the tonsil, it might be well to gain some idea of its function. This, however, is a difficult matter, for to say the least the nature of the activity of the tonsil is by no means clear. Early observers believed that the tonsil secreted mucus to help lubricate the bolus of food in swallowing, but later it was shown that the tonsils are devoid of mucus glands in their parenchyma. The mucus ducts of the faucial region do not open into the tonsil, but empty directly into the buccal cavity at their periphery. While the tonsil does not secrete mucus, there may nevertheless be an escape of serous fluid from its tissue into the mouth.

At the present time it is believed by many, that the tonsil has a function similar to that of a lymph node. Fox, in 1885, pointed out that the tonsil did not secrete, but that in reality it absorbed materials from the mouth. In support of this a number of observations have been made to prove that the tonsil has the power of absorbing foreign particles from the mouth. Goodale found that carmine pigment, which had been injected into a tonsil crypt, would be absorbed, and Wright has demonstrated that soot particles (carbon) find their way into the tissues of tonsils in children who have been living in the smoky atmosphere of Pittsburgh. Experimentally the production of tuberculous lesions of the tonsils by swabbing emulsions of tubercle bacilli on their surfaces, has been advanced as evidence of their ability to absorb foreign particles. Hodenpyl, however, took exception to this view, believing that the tonsil had very little power of absorption. It is obvious that the invasion of a tissue by any type of bacterium is no criterion as to the function of absorption of that particular tissue.

Histologically it has been pointed out that the tonsil strongly resembles a lymph node. At first sight, this appears to be true, but on closer analysis it is found to differ considerably from a lymph node. The tonsil is devoid of the large afferent and efferent lymph sinuses that constitute such important parts of the lymph glands. The efferent channels have their analogues in the tonsil in the shape of the fine canaliculi which empty into the crypts. But this system is quite different from that present in the lymph nodes, where the efferent vessels have a direct communication with the lymphatic circulation.

In consideration of its anatomical structure and position it is probably best to consider the tonsil as a part of the general lymphoid structures that are present throughout the whole alimentary tract. It is noted that, especialy in the pharynx, small lymphoid collections are numerous, while the Peyers patches and solitary follicles of the intestine need only be mentioned as being of the same type found along the same canal. In young children collections of lymphoid cells are not uncommon and often very prominent in the stomach. This whole system of lymphoid structures is believed by many to have an important relation in the etiology of various infectious diseases. As we have referred to above, Goodale and Wright believe that pigment particles are taken in by these lymphoid structures. In this connection we were surprised to find that in the examination of all of our tonsile we were able to indicate the presence of a single mass of carbon pigment in a large phagocytic endothelial cell lying near the margin of a tonsillar crypt in only one case. The pigment was made out by the oil immersion lens, and even here we have a reasonable doubt as to whether it was truly carbon. As we have made no special study of the adenoids, we cannot say whether or not they actively absorb pigment, but by analogy we would expect to find no more in them than was obtained in the tonsil. From my own observations, however, I feel quite justified in saying that the tonsil has not the power of taking up carbon pigment from the inspired air, and moreover since practically all of these cases have lived in a vicinity in which the air contains from time to time a considerable amount of carbon, these observations are therefore more striking. We know from our autopsy findings that the peribronchial glands and lungs in this district contain an enormous amount of this pigment, and that it would be present in the tonsil if that structure had any selective action in picking it up. Recently, in one of our autopsies on a coal miner whose lungs and peribronchial glands were intensely pigmented by carbon, we were unable to find any pigmentation of the tonsils. The fact that the large endothelial leucocyte is of a minor importance in the phagocytic activity of the tonsil, may help to explain this negative finding, since it is this type of cell which is chiefly concerned in taking up carbon in the lungs.

In a general consideration of the tonsil, it would appear a quite unnecessary function, that debris and material should be obsorbed from the open buccal cavity. These substances serve no useful purpose in the animal economy, and it would indeed be strange that nature should burden the system in undertaking a useless work. The mouth is much more readily cleansed by the secretions which flow into it, some of which are bactericidal.

The injection of pigment into the crypts of the tonsil we believe is not a reliable method to demonstrate the absorbing property of this organ. The element of trauma to the lining cells by the experimental procedure is a factor leading to an inflammatory reaction, which must be considered. Naturally, where the lining cells are broken absorption and phagocytosis will occur on the surface. This view was appreciated by Wright who considered that the finding of soot particles in the tonsils of children living in the smoky atmosphere of Pittsburgh was a much more valuable indication of the absorbing power than if the pigment had been painted on experimentally. Wood, by injecting pigment was able to follow the lymphatic route from the tonsil to a gland belonging to the deep cervicle group, under the angle of the jaw. This he termed the tonsillar gland. If carbon pigment were constantly absorbed by the tonsil and carried to the lymph nodes draining it, we should certainly see pigmentation of the upper cervical nodes. Autopsy findings and surgically removed cervical glands indicate otherwise, for carbon pigment is never found in these structures. We are therfore led to believe that the normal tonsils do not absorb solids.

As we shall indicate in the section on the anatomy, the tonsillar cells appear to be constantly moving toward the crypts and the cells of the parenchyma, the follicles and interfolliclar tissue, are seen in the crypts and constantly in the narrow spaces between the epithelial cells lining the crypts. This is known as Stöhr's phenomenon. That there is some process of filtration or other physiological activity occurring in the tonsil and that some cells are broken up and discharged into the crypts, we consider as very probable. In considering the filtering function of the tonsil, the question arises, how are the materials brought into contact with parenchyma of the tonsil. From our observations we believe that solids are not absorbed by the tonsil, and regard our own negative findings of carbon pigment and other solids as strong evidence for this contention. The only apparent explanation is that the tonsil is able to absorb solids in solution and thus bring them in contact with the parenchyma. On the other hand the possibility of the tonsil receiving afferent vessels which have collected materials from other regions of the mouth must also be admitted. There is no evidence to indicate that the tonsil has an essential internal secretion, in fact the clinical findings, that their removal causes no systematic disturbances indicate quite the opposite. If the normal tonsil can absorb fluid, which is quite probable, but of which we have no definite evidence, then the same function must also be ascribed to the other lymphoid structures in the pharynx, for it would not be in keeping with the anatomical facts to ascribe to the tonsil the role of being the only channel by which material from the mouth might enter the circulation.

Quite a controversy has arisen between several concerning the importance of the 'physiological wounds' in the tonsil as described by Stöhr. Some (Levinstein, Breiger, Lindt and others) have indicated that these channels are of value in permitting cells having bactericidal properties to escape onto the surface of the tonsil. On the other hand Schoenemann considers these as points of absorption and that phagocytic activity of the organ takes place in the parenchyma. Renn has observed that the type of cell which is exuded into the crypt by way of the 'physiological wounds' bears some relation to the processes in the tonsil. Under normal conditions the lymphocytes are found in slight excess over the leucocytes in the exudate. This proportion is reversed with the onset of inflammation.

ANATOMY OF THE TONSIL.

The faucial tonsils are two symmetrically placed prominent bodies situated between the anterior and posterior pillars of the soft palate, and covered over by a layer of epithelium which is continuous with that of the mouth cavity. The prominence of these bodies varies at different ages, and even in individuals of the same age. We have found that the average size of the tonsil we received was 2.5x1.8x1.5 cm.

The tonsil is bounded by muscular bands on three sides. The palato-glossus forms an arc along the anterior border, the palato-pharyngeus lies posteriorly, while the constrictor pharyngeus superior passes externally. Thus although the tonsil possesses no muscle bundles of its own, there are small strands from these muscles inserted into its capsule, which may be able to alter its position or possibly even to compress it.

Each tonsil is enclosed in a fibrous capsule which completely surrounds it, save on its inner surface, where the openings on the mucosal surface are seen. The capsule has its origin in the aponeurosis of the pharynx. Posteriorly and externally the capsule and the aponeurosis are so closely allied that they cannot be distinctly separated, but as the mucous surface of the mouth is approached the outline of the capsule becomes quite clear. On the inner and free surface of the tonsil the capsule forme a very thin membrane which lies beneath the mucous membrane in place of the sub-mucosa. In those places, however, where a crypt opens on the surface, the capsule thins out and is gradually lost in the stroma of the tonsil. Thus the capsule gradually diminishes in size as it extends anteriorly. In the capsule and pericapsular tissue, which is the somewhat looser connective tissue beyond the tonsil, there are several anatomical structures that are almost always present, blood and lymph vessels, muscles, mucous glands and ducts. We will discuss the vessels later, and at the present confine ourselves to the last three structures.

The muscle bundles in and about the capsule are quite numerous and are best seen in the posterior parts and in the pericapsular tissue, while externally and anteriorly they are fewer in number. Again occasionally muscle bundles are seen in the trabeculae within the tonsil but very rarely in the tonsillar substance itself. These last positions of the muscle fibers are abnormal and we will refere to them again in discussing the trabeculae. The muscle fibres are of the striped variety, and are associated with the neighboring muscles as we have indicated above. Toepfer has pointed out that many of the muscle fibres are constantly to be found inserted into the capsule of the tonsil, and he speaks of them as the "musculi tonsillares." As regards any special action of these muscles on the tonsil, it is difficult to speak with certainty, and we have been unable to find any definite reference to it in the literature although some suggest that the muscles tend to compress the tonsil, and in this way to empty the crypts.

Surrounding the capsule there is a chain of mucous glands which is not, however, observed in all cases. Usually one can find numbers of active glands having more commonly a lateral and anterior position to the tonsil. These glands never enter the tonsillar substance, but occasionally they are to be seen in the trabeculae. This must be considered as an abnormal position for them. Mucous ducts from these glands are of course present. The ducts which lead from the mucous glands open on the mucosa of the mouth beyond the area occupied by the tonsil. We have never been able to find a duct opening into a crypt of the tonsil. Not infrequently, however, do we see ducts lying in the trabeculae and even in the tonsillar tissue close to the true capsule. These ducts have apparently taken an abnormal course, or they may be associated with the aberrant mucous glands previously mentioned; but their openings into the mouth, we believe, are always outside the tonsil and never into the crypts.

The mucous surface covering the tonsil varies considerably in appearance. It is usually convex, but it may at times be concave. As a rule in the young it is convex, while in adults the 'hidden' or concave surface is commonly observed. The surface may be smooth or ragged, depending upon the character of the mouths of the crypts. If, therefore, the openings of the crypts are widely patent and funnel shaped, the buccal surface is irregular. Inflammation, either acute or chronic, of the tonsil will influence very much the appearance and regularity of the mucous surface over the tonsil.

The crypts of the tonsil are diverticula of the buccal cavity which are lined by squamous epithelium. They are from twelve to fifteen in number and open freely into the mouth on the mucous surface of the tonsil. The crypts extend into the tonsil almost to the capsule, where they end blindly. The contents of the crypts, therefore, are to a large extent the same materials as are found in the recesses of the buccal cavity, that is saliva, food debris, cell debris and bacteria. From the main crypt smaller branches are given off.

These finer crypts are at times quite narrow and extend for a considerable distance into the tonsillar substance, while at times they have been observed to enter the centre of a follicle. They are very important in their relation to inflammation. The cavity of the crypt is normally small but in the presence of inflammation its lumen readily changes. The lining of the crypts is composed of a thick layer of squamous epithelium continuous with the lining of the mouth. It differs from the buccal lining in that the basement membrane is usually absent in the deeper portions of the crypts. This, however, is not always true for in some crypts a definite basement membrane can be observed beneath the entire epithelial lining. The superficial cells are flattened and may become keratinized. The deeper epithelial cells are larger, rounded, and extend directly into the tonsillar substance, so that it is often difficult to make out where the epithelium stops and the parenchyma of the tonsil begins. These deeper epithelial cells are morphologically very similar to the large cells seen in the centre of the follicles. This appearance is brought out more forcibly in some sections where we have observed prolongations of the epthelial lining, extending deeply into the tonsillar substance and having at their extremity a collection of epithelial cells, which when observed alone, one would unhesitatingly call a follicle.

Another point of interest in the lining of a crypt is the presence of small channel-like spaces which extend through the epithelial layer and open into the cavity of the crypt. Stöhr refers to them as ''physiological wounds,'' and lays a great deal of stress on their importance as the routes of tonsillar infection. They are small slit-like openings between the epithelial cells which open at one end into the crypt and at the other end are lost in the deep tonsillar tissues. We have never observed a ''physiological wound'' which presented a communication between the lymphoid follicle and the crypt. These physiological wounds are not lined by endothelium, and in this they differ from lymphatics with which they have been compared. In these spaces are observed cells of different types, most commonly polymorphonuclear leucocytes, mononuclears, and cells of the follicles, while we have rarely found cells of endothelial character. By ''cells of the follicles'' we refer to the large mononuclear cells often showing nuclear division, which are found in the central part of the follicle, and as far as we have observed have their origin in that situation. These cells of the follicles are by no means as frequent invaders of the ''physiological wounds'' as the other two types. The mononuclears are cells of a lymphoid character and probably arise from the large central cells of the follicles. In the inflammatory conditions these channels are dilated and the polomorphonuclear leucocytes predominate. The physiological wounds do not contain red blood cells. We regard these structures not as definite and fixed channels, but merely as spaces between the epithelial cells caused by the outpouring of cells from the parenchyma of the tonsil. The cells which are thrown off wander toward the crypts.

Coming now to the structure of the tonsil proper, we must consider in turn the trabeculae, stroma, follicles, the blood and lymph supply, and the individual cells of the tonsil.

The trabeculae are fibrous bands arising from the capsule. They extend across the tonsil and are gradually lost anteriorly by becoming united again to the capsule under its mucous surface, or else, they disappear in an aborescent manner in the stroma of the tonsil. Sometimes these bands are large and broad throughout their course, dividing the gland into alveolar masses. The trabeculae carry the large vessels and lymphatics, and give origin, in part, to the stroma of the tonsil. The vessels in the trabeculae are quite large and in studying them one wonders why severe bleeding is not more common in tonsillar surgery. Aberrant structures as muscles, mucous glands and ducts are occasionally seen in the trabeculae, particularly in the region of the capsule. These have been previously mentioned so that we need merely refer to them. In some (about ten per cent of cases) of our sections muscle bundles were seen in the central part of the tonsil in the trabeculae and recently a large muscle bundle was noted in the tonsillar substance proper lying between the follicles, without having any relation to the trabeculae. This was the only instance of this kind in our series of cases. Toepfers explanation, that the muscle bundles in the trabeculae are aberrant tissues, is clear and simple. Many of the small muscles of the pharynx have a slight insertion into the capsule of the tonsil, and in some instances, some of the fibres extend more deeply along the trabeculae and are carried into the tonsil. The stroma of the tonsil is derived from the capsule and from the trabeculae. It is a loose connective tissue structure forming the supporting framework for the tonsil cells. Under normal conditions the stroma exists as a very delicate reticulum which is very indistinct in ordinary sections.

The follicles make up the greater part of the tonsillar substance proper. They consist of numerous large nodules or collections of cells which usually arrange themselves around the terminal crypts.

In size they vary greatly. The follicles of the tonsil are similar in cellular structure to the follicles of the lymph nodes. The cells in the center of the follicles are large with prominent nuclei and abundant protoplasm, and are very like the deep epithelial cells of the lining of the crypts. These cells usually make up about one-third of the follicle. At the periphery of the follicle the cells are smaller with deeper stained nuclei and have less protoplasm. They are not unlike lymphocytes and probably represent a prelymphoid cell. A number of cells showing nuclear division are always found among the central cells, and a few in the periphery. These cells are large and belong to the same order of cells as those in the centre of the follicle. Endothelial cells showing phagocytic characters are noted in the follicle but they are never numerous. The interfollicular tissue is made up of a very loose reticular stroma containing many fine blood and lymph capillaries, and packed with mononuclear cells which are chiefly of the prelymphoid and lymphoid type. Besides these, however, the large central cells of the follicles, some of which show nuclear divisions, are to be noted passing toward the crypts. Plasma cells are always present while eosinophiles are scarce. In a recent paper Davis has shown that plasma cells appear about the second or third week after birth, and from that time on are to be constantly found in the tonsil. Wandering phagocytic endothelial cells are never numerous.

The blood vessels of the tonsil enter through the outer capsule and are distributed along the trabeculae where they give off smaller branches, which enter the soft tissues and break up into capillaries. These capillaries are very numerous especially in the inter-follicular tissues, and obviously they become still more prominent in inflammatory conditions. Their lining endothelial cells are as a rule well marked.

The lymphatics of the tonsil, we believe, bear the same relation to the tonsil as do the lymphatics to any organ. They follow a course similar to that of the blood supply, the large trunks lying in the capsule and trabeculae, while the capillaries extend throughout the soft tissues. There are no large afferent lymph sinues or system of sinusoids in the tonsil, so that in this point the tonsil bears no comparison with the structure of a lymph gland. Around the follicles the lymphatics have a concentric arrangement. It has been previously pointed out by Goodale and others that the tonsil has no true afferent lymphatic system but that the ''physiological wounds'' may act as such. We do not agree with this contention. We believe that the tonsil like other organs has both an afferent and efferent system of lymphatics, but that this system is only a passive drainage for the organ. As regards the relation of the physiological wounds to the tonsil in general, and more especially to the lymphatics, we have made no particular study. We believe, however, that in view of the fact that the physiology wounds contain cells and the debris of cells, which are derived from the tonsil, these spaces have marked efferent functions, while there is no evidence suggesting an afferent function to these channels. There is nothing to indicate that the physiological wounds are in any way associated with the lymphatic channels of the organ.

Wood has shown that the general lymphatics of the organ drain into a gland at the angle of the jaw. This he calls tonsillar gland. This gland he believes belongs to the group of deep cervical lymphatics, which drain toward the thorax.

INFLAMMATION OF THE TONSIL.

The tonsil is probably more frequently subject to acute inflammation than any organ in the body. It is evident, in fact, that those who have escaped one or more attacks of tonsillitis may be regarded as the exceptions. The explanation of the frequency of this disease is fairly clear, and may be considered under two heads; firstly, the prominent position of the tonsil in the body, and secondly the anatomical structure of the tonsil. The latter reason is certainly more important, but at the same time, is in part, dependent upon the first.

The tonsil, lying as it does, in the walls of the buccal cavity, has a most intimate relation with it, and therefore, comes in direct contact with all types of bacteria which may infest the mouth. Its anatomical position then, affords ample opportunity for infection. On the other hand, the very structure of the tonsil constitutes an important factor for bacterial invasions. Under the anatomy of the tonsil we have previously pointed out that the crypts are merely diverticula of the buccal cavity extending into or rather surrounded by tonsillar substance in the nature of the follicles. Obviously the contents of the crypts is similar to that of the mouth, as bacteria, food debris, epithelium and mixed secretions. The crypts, which extend deeply into the tonsil, have a number of finer branches running from the main trunk. In these finer branches, and in the deeper portions of the large crypts, the circulation of the contents toward the surface must necessarily lag, and the bacteria-containing materials tend to collect. This stagnant material may, and often does, act as an irritant and set up inflammation. Moreover, some of the crypts are sac-like in shape, having a narrow neck with a dilated body below. Such a type is particularly liable to accumulate harmful products. When we consider that the tonsil contains from eight to sixteen of these crypts, it is seen that the opportunity for injury and infection is very great.

It is evident, therefore, that the inflammatory processes of the tonsil have their origin in irritants which invade the organ from the mouth. In the majority of instances the lesions are due to the direct action of bacteria, and of these microorganisms the streptococcus pyogenes is the commonest invader. However in carefully studied cases indicating acute tonsillitis, organisms of the type of Micrococcus salivarius or Micrococcus Rheumaticus may be found. To what extend, the organisms found associated with the streptococcus play in these infections, in bringing about the lesions in the tissues, cannot be stated.

On the other hand it is well to remember that in a few instances the infection of the tonsil is metastatic in nature. Secondary syphilitic lesions of the tonsils in which spirochaetes are readily demonstrated, are types of blood infection. Tuberculosis of the tonsil has also been indicated to be metastatic, either by way of the blood or lymph stream. Examples of metastatic pyogenic infection, we have not encountered.

Acute inflammation of the tonsil may be divided into three divisions: (1) acute lacunar tonsillitis, (2) acute parenchymatous or true follicular tonsillitis, (3) acute peritonsillitis. This classification is the one suggested by Semon and Williams and in our opinion it is the most useful one.

Acute Lacunar Tonsilitis.—Acute lacunar tonsilitis is an inflammation confined entirely to the lining of the crypt, and not extending deeply into the substance of the tonsil. The condition is commonly spoken of as follicular tonsillitis on account of the white plugs of material appearing at the mouths of the crypts. The process usually begins in the deeper and dependent portions of the crypts with an erosion and destruction of some of the superficial lining epithelial cells. A broken surface is produced and the bacteria which are present gain an entrance at this point. Local congestion and infiltration by polymorphonuclear leucocytes soon takes place and we have the formation of the ulcer. This is analagous to the ulcer formation in the appendix described by Aschoff. The ulcer may and frequently does remain superficial, but it may also extend deeply and invade the tonsillar substance by destroying the epithelial lining. This would appear contradictory to what has been previously said, that in this type of inflammation only the lining of the crypt is involved; but we must remember that the acute inflammatory process of the crypt, if the infection is severe, can easily extend beyond the epithelium into the surrounding tissues. As a rule the ulcer is small and localized, and is not distinguished macroscopically. With the relatively enormous outpouring of the polymorphonuclear leucocytes at the point of ulceration we find the crypts become distended with them and that these cells wander much beyond the ulcerated area, giving rise to soft plugs of exudate which may sometimes be seen at the mouths of the crypts. This point is of interest and importance because one may quite frequently observe a crypt packed with polymorphonuclears, while its lining epithelium appears intact and smooth. Undoubtedly in these sections we have entirely missed the point of ulceration but are not beyond the exudate in the crypt adjacent to the ulcer. A number of sections from the same block, or better still, serial sections of such tissue overcomes this difficulty. In our series we were very much surprised to find the large number of tonsils which showed acute inflammation in the crypts. In many cases we were able to demonstrate the ulceration, but frequently our sections showed only the polynuclear infiltration of the crypts. When it is considered that the great majority of these tonsils, which we examined, were removed on account of recurrent attacks of tonsillitis with subsequent hypertrophy, but never were they removed during the clinical stage of acute follicular tonsillitis with its general symptoms, it was of interest to us to find acute inflammation with ulcers in the lining of the crypts so frequent. It was present in fifty-five per cent of all our cases. Hence we believe that acute lacunar inflammation is a fairly constant pathological lesion in those having recurrent attacks of tonsillitis and that it may exist without marked systemic symptoms, which are usually seen in acute follicular tonsillitis. Moreover it has not been an infrequent finding in those cases which clinically presented a condition of hypertrophy of the tonsil.

Polymorphonuclear leucocytes, the chief cells to be seen in acute lacunar tonsillitis, are found closely packed in the crypts around the ulcerated area. These cells are undoubtedly the main phagocytic cells of the tonsil. This has previously been noted by Goodale and other observers. That the polymorphonuclear leucocytes are phagocytic for bacteria can be readily shown by opening a crypt and making smears from its contents. Small chains of three or four cocci and occasionally bacilli were noted in them, but single and diplo-cocci were more commonly found. A number of polynuclear leucocytes constantly break down and with the desquamated epithelial cells and other debris form a considerable amount of necrotic material. Throughout this plug of exudate we very often found fibrin, which in some cases was much more marked than in others. Strands of fibrin are also seen attached to the margins of the ulcer. This variable quantity of fibrin is a part of the tissue reaction in the inflammatory process.

The large endothelial leucocytes were not plentiful and although they were phagocytic when present they did not appear to play a very important role in the acute inflammatory reactions. The same might be said of the lymphoid cells as regards phagocytosis, and we have no evidence in our series to show that they ever act as such. They were usually present only in small numbers in the exudate.

In view of the above findings it is quite apparent that in this form of tonsillitis in which the surface lining of the crypts is destroyed, one of the chief barriers to infection is broken down, and ready access is gained by the bacteria to vulnerable tissues. In this type of tonsillitis alone, did we find septic organisms in the deeper tissues at the margin of the ulcer. It is obvious that such an ulcerated surface as above described, offers a ready portal of entry for other organisms, which are not found in the normal tonsil. Moreover it is probable that such acute lesions precede the more chronic tuberculous infections. In fact it is highly probable that the tubercle bacillus is only able to gain entrance into the tonsil by such ulcerating surfaces.

It is a well known clinical observation that tonsillitis or sore throat frequently precedes or accompanies an attack of acute rheumatic fever, and hence the term rheumatic tonsillitis has at times been applied to this throat affection. However, it is quite evident that the majority of cases of tonsillitis are not followed by rheumatism, and further that many cases of acute rheumatic fever are in no way (clinically) associated with acute lesions of the tonsils.

If we infer that all cases of tonsillitis, which are immediately associated with rheumatism, indicate the tonsil as the route of infection, then those cases of tonsillitis unaccompanied by rheumatic fever which form the vast majority of tonsillar inflammations, must necessarily suggest one of two things. Either the etiological factor of rheumatic fever is not present in the tonsil crypts, or else it is present only in small numbers or is so attenuated that the phagocytic cells of the tonsil can easily destroy it.

We have observed that when symptoms of acute tonsillitis are present there is always a pathological basis as shown by ulceration of the lining of the crypt. We, therefore, feel that a similar lesion is present in the tonsillitis preceding rheumatism, and as far as we know, this lesion differs in no way from that of acute lacunar tonsillitis.

To repeat what has been said elsewhere, we regard the ulceration of the lining of the crypts as being the vulnerable point of entry of organisms from the mouth. It matters little apparently what bacteria have produced the lesion, for once the ulcer has formed it is possible for any type of organism to gain access to the deeper tissues. Such is the probable origin of the acute rheumatic fever following tonsillitis. The tonsillitis is not rheumatic but pyogenic in origin, and the development of rheumatism is in all probability due to an over whelming invasion of the rheumatic infection, the nature of which is not definitely known.

We must also remember that this infection may enter through other portions of the alimentary tract, as the mucous ducts in the pharynx, and by way of the lymphoid structures of the small and large intestines. The determination of the etiological factor of acute rheumatic fever will also tend to make plainer the routes of invasion of this disease.

The bacteriology of the tonsillar crypts has been repeatedly determined so that we shall merely refer to it. The organisms found in the tonsil are in general the same as those of the buccal cavity. Streptococci, staphlococci, pneumonococci, micrococcus catarrhalis, and the diphtheria group are the important organisms. In properly stained sections these and other bacteria appear very numerous in the exudate of crypts.

Acute Follicular Tonsillitis. An acute inflammatory process involving the cellular follicles of the tonsil is an acute follicular tonsillitis. This is not necessarily the clinical form of follicular tonsillitis, for the latter condition is chiefly diagnosed by the presence of an exudate in the crypts, and, therefore, may only constitute an acute lacunar tonsillitis. In the follicular tonsillitis here under discussion the inflammatory process is within the substance of the organ, and having also more or less acute exudate in the crypts. The relation between acute lacunar and this type is very close, as the acute follicular is probably always secondary to a cryptic inflammation. As we have indicated under the anatomy of the organ, fine branches of the large crypts were sometimes noted in the central part of the follicles suggesting that there was a definite relationship and communication between the follicles and the epithelial invaginations. In inflammatory conditions this relation is again brought out but in quite a different way.

In some cases having an acute lacunar inflammation a slight but definite change was noted in the parenchyma of the tonsil. The change was localized to the follicles bordering on the crypts and consisted of small collections of polymorphonuclear leucocytes and some necrosis occurring in the central part of one or more of them. Where these leucocytic collections were small the periphery of the follicle was quite clear and normal looking; but in others the whole follicle was infiltrated and looked not unlike a small localized abscess. The tonsillar substance otherwise was clear. Here we have an early infection of the tonsillar parenchyma extending from the crypts and beginning in the central portions of the follicles. As the infection proceeds, the follicle and then the interfollicular tissue may become involved. We have noticed, in some cases, that follicles at some distance from each other showed about the same degree of acute inflammation. The interfollicular tissue was quite free so that it appeared improbable that the infection had traveled from one to the other. In these cases we believe, that the infection independently reached the follicles from the crypts. Thus in acute follicular tonsillitis we may have any number of follicles involved depending entirely upon the character and severity of the infection in the crypt and also upon the number of follicles bordering upon that crypt. The infection first shows itself in the central part of the follicle and from here it may extend into the interfollicular tissue. We admit that in severe infections it would be possible for one follicle to be involved by the direct extension of the infection from others but in milder cases, and of these we have had a number, the interfollicular tissue has always remained uninvolved suggesting that the route of the infection was from the crypt to the follicle. The amount of tonsillar substance affected by the inflammatory process bears a definite relation to the general systematic symptoms. As we mentioned above, acute lacunar tonsillitis is always present in follicular tonsillitis and with the enormous outpouring of leucocytes the crypts are filled with exudate which is seen as yellowish white dots at their openings on the mucosal surface of the mouth.

The tonsil is richly supplied with blood vessels, and consequently in acute inflammation when they become engorged the polymorphonuclear leucocytes are found passing through and surrounding the walls of the vessels. This abundant supply may afford an excellent opportunity for infection gaining a rapid entrance into the general blood stream. It may be that the phagocytic leucocytes assist the bacteria in converting a local into a haematogenous infection. Hence systemic symptoms are as a rule rapid, and according to the nature of the infection vary in severity.

Again in this form of acute inflammation the polymorphonuclear leucocyte is the principal phagocytic cell. The endothelial cells, although seen quite frequently in follicular tonsillitis, appear to be of minor importance in the phagocytosis of bacteria.

The presence of fibrin in the follicles, showing no other evidence of acute inflammation but a rather poorly staining central zone, was frequently noted. Many of the follicles in different sections of the same tonsil have been shown this. The indication here is that a previous and fairly recent attack of acute follicular tonsillitis had occurred and fibrin had been laid down, which when seen was in the process of absorption. Or, too, it may be, that the cells of the tonsil had suffered the effects of a toxin which did not react by immigration of leucocytes, but by the deposition of fibrin. During the absorption of the fibrin the endothelial leucocytes may play an important phagocytic role as they are more commonly found in the follicles than elsewhere. We have observed no evidence of organization by connective tissue of any acute lesions within the follicles.

We have been unsuccessful in demonstrating bacteria within the tonsil tissue proper, save in the immediate region of an ulcer or abscess. Bacteria, have however, been demonstrated by other observers, and no doubt during the very acute stages of follicular tonsillitis they are present in large numbers.

Acute Peri-Tonsillitis.—Acute peri-tonsillitis or what is commonly known as quinsy is an acute inflammation in the capsular and pericapsular tissue of the tonsil. This inflammation is supposed to arise from an infection passing from a primary focus in the tonsil to the peri-capsular tissues. There is, however, no definite evidence to indicate that the infection comes from this source, while we have found that the majority of infections come by another route.

The numerous ducts of the mucous glands which are practically always present in the peri-capsule tissue, open into the mouth beyond the border of the tonsil. These ducts form open channels from the mucosal surface to the gland structures which surround the tonsil on all sides. Consequently, when an infection takes place through these channels the resulting inflammation will be independent of any process within the tonsil proper. We have not had an opportunity of studying this condition in excised tissues with extensive inflammation involving the ducts, but at the same time, we have frequently observed a marked infiltration of lymphoid and plasma cells around them, indicating a chronic inflammation. We have also observed the presence of inflammatory exudate within these ducts at varying distances from the buccal surface, without any inflammation within the tonsil. From these repeated observations we have concluded that an ascending infection through these channels takes place, and that under certain conditions an acute inflammation of the ducts and mucous glands gives rise to a suppurative process of the peri-tonsillar tissues.

Microscopically the infection is characterized by a marked polymorphonuclear leucytic infiltration into the loose tissues around the tonsil with a marked out-pouring of serum. The tissues of the parts rapidly break down with the formation of an abscess, which usually presents on one side of the tonsil. The infection as a rule is of a streptococcic nature, which in the chronic stages may become mixed.

Chronic Inflammation of Tonsil.—It is evident from what we have said under acute inflammation that almost every individual is subject to one or more attacks of tonsillitis during life time. In some individuals this inflammation is repeated at varying periods, so that a greater or less inflammatory reaction takes place in the tonsil quite frequently. In fact it is not an unusual clinical observation that some individuals have a constant slight inflammatory reaction in the tonsillar tissues, which becomes aggravated and allow seasonal irritants to produce a very acute process. To the clinician these recurrent inflammations of the tonsil represent a chronic form of the disease. From a pathological view, however, the examination of the tonsils in such cases would, during any of the periods of the exacerbation, present all the characters of the acute condition superimposed on chronic tissue changes.

In this discussion on chronic inflammation of the tonsil we refer, not to the clinical form which is gauged by the time that an inflammatory condition has existed in the tonsil, but to the actual changes that are to be observed in the tissues, and which we recognize as a condition of chronic inflammation. Generally speaking, chronic inflammations refer to the end results of the stages of healing of an acute inflammatory infiltration. At times this is to be recognized by the presence of lymphocytes, plasma cells, eosinophiles, fibroblasts or connective tissue. The development of new blood vessels in the lesion is also an evidence of the acute condition passing into the healing or chronic stages of the lesion, while changes are also to be observed in the crypts and their lining cells.

It is possible, but we believe in connection with the tonsil not probable, that chronic lesions may have developed by the presence of a very mild irritant without having had the presence of a previous acute inflammation. We are familiar with such conditions arising in other organs, but, as we have previously indicated, many of the conditions of the tonsil, which clinically are not recognized as inflammation can be demonstrated to possess an acute lesion when it is properly looked for.

As the majority of the tonsils which we have examined had been removed during the interval of recurrent attacks, and never in the acute inflammatory stage as we see it clinically, it was expected that a fair number would show the histological characters of the chronic disease. It was a surprise to us to find how frequently acute lacunar inflammation was associated with many chronic forms of tonsillitis, in these cases with recurrent attacks.

If chronic inflammation of the tonsil bears a close relationship to the acute conditions, the situation of the lesions will be quite similar or identical in these two types. We shall discuss the chronic inflammation of the tonsil under (1) Chronic Lacunar Tonsillitis, (2) Fibrosis of the Tonsil, (3) Chronic Peritonsillitis.

Chronic Lacunar Tonsillitis.—Just as acute inflammation was observed to be most frequently localized to the crypts and their lining tisue, so do we also find the commonest form of the chronic inflammation situated there. In chronic lacunar tonsillitis a number of changes are to be seen in the lining and in the size and contents of the crypts.

In the normal tonsil the outline of the squamous lining of the crypt is comparatively regular and smooth. A number of fine crypts with small openings pass downward from the main crypts but do not cause much irregularity on the outer surface. In this form of inflammation, however, the epithelial lining tends to become irregular, due chiefly to a dilatation of the finer branches of the main crypt, and also to the fact that the epithelial cells are more widely separated, as the spaces between them are packed with cells which are migrating into the crypt. Consequently if the irritation becomes constant or chronic, more tonsillar cells are thrown off, and greater irregularity in the lining epithelium is the result.

Not infrequently the lining assumes a papillomatous appearance which may become so pronounced as to be easily recognized in the gross specimen. These projections are similar in structure and formation to papillomata of squamous epithelium found elsewhere in the body. They are covered by a thick layer of squamous cells, which at times keratinize and have a loose fibrous stroma usually containing a few lymphoid cells. Chronic inflammation with its various tissue changes is undoubtedly the important etiological factor in their production.

In chronic inflammatory diseases of the tonsil the epithelial cells themselves undergo considerable change. The superficial lining cells show marked keratinization and are freely desquamated into the lumen of the crypt, and may eventually completely fill it. Occasionally types of epithelial pearls are to be seen. It is considered by some that the finding of epithelial pearls in the tonsils represents the remains of epithelial islands present in the embryonic structure, and that these islands are similar to Hassall's corpuscles found in the thymus. We would point out, however, that the finding of these concentric masses is associated with the chronic tonsillitis, and that they represent the accumulation of desquamated epithelial cells due to partial occlusion of the mouth of the crypt. Ulcers in the various stages of healing are also to be met with in the lining of the crypts. The more superficial ones heal with very little scar tissue, and it is often impossible to say whether ulceration has been present or not. However in the more severe, and also in the more recent ones, new formed capillaries, dense lymphoid infiltration with a loss of the epithelial lining can easily be made out. A few polymorphouclear leucocytes and phagocytic endothelial cells are to be seen in the healing ulcerations.

A number of changes are to be noted in the crypts and their contents in chronic lacunar tonsillitis. The cavity of the crypt is usually dilated, extending to the opening into the buccal cavity, and giving rise to widely gaping mouths on the mucosal surface. This, however, is not always true, for the mouth of the crypt may be very small or even closed, while the deeper portions of the crypt may be much dilated. Thus one cannot tell from the appearance of the mucous surface, the condition of the deeper portions of the crypts. This alone indicates how difficult, in fact, in some cases how impossible, it would be to reach the confined material in the crypts by ordinary methods of treatment.

The contents of the crypt in this condition is chiefly desquammated keratinized epithelium along with a small number of lymphoid cells. Endothelial cells are very few in number, and even when lying free in the crypt do not appear to take up carbon pigment. If an acute lacunar inflammation is associated, which is often the case, polymorphonuclears are present. It is also in this condition that we find a type of mycelial growth so commonly seen in the tonsil. Symmetrical enlargement of the lumen of the crypt was noted in many cases, so that the crypts appeared as large oval or round almost cyst-like spaces. The lining as a rule was quite smooth, and considerably compressed. In places it was thinned to two or three layers of flattened cells. The contents were composed of homogeneous pink straining material which almost invariably was free from leucocytic infiltration. The homogeneous material often showed peculiar slit-like clefts as are seen in areas containing cholesterin. The crystals of cholesterin could not be recovered from the paraffin sections. In both the fresh and formalin fixed material crystals of cholesterin are numerous and were easily demonstrated. That the homogeneous contents is composed of debris of keratinized epithelial cells is quite easily shown, especially if a crypt in the early stages of dilatation is examined, where the cells are in the process of desquamation. The formation of cholesterin follows the breaking down of these cells, and does not arise from any form of tonsillar secretion.

The shape of these dilated crypts is very suggestive that there has been a blockage of the outlet, and the compressed lining strengthens this view. Thus we have to do with a type of retention cyst. In two cases we noted that the contents had broken through the wall of the sac and had invaded the tonsillar tisue, but even in these cases we could not demonstrate any leucocytic infiltration or other evidence suggesting inflammation as a consequence to this rupture. It is probable that this was caused by trauma at operation. Only rarely have we been able to show the large foreign body giant cells which are not infrequently associated with these crystals in other tissues. Their absence is probably due to the fact that the lining of the crypt is quite smooth and firm and does not give an opportunity for the cholesterin to act as an irritant; consequently giant cells do not tend to form. In the crypts where the opening is patent and where there is a good deal of keratinization of the lining epithelium, cholesterin was also found to be present.

In the gross specimens the dilated crypts containing cholesterin are easily made out and at times the molded mass of debris forms nodules, as large as split peas. The material is soft, yellowish-white and is not unlike sebaceous material. Cholesterin crystals in the form of the characteristic plates can be readily distinguished in direct smears.

In a few of the dilated crypts, containing cholesterin, calcium salts have been noted. The accumulation of these salts indicate the early development of tonsillith. These calculi vary considerably in size and may become quite large and hard. Robertson reported one weighing one ounce. It has been advanced that they are of gouty origin but urate of soda is rarely, if ever, seen, and Robertson has shown that calcium and magnesium salts are the inorganic substances present. As to their origin there is some difference of opinion. Semon and Williams believe that Leptothrix Buccalis (mycelial growths) formed their nuclei and with the asociation of bacteria, mucus, and broken down cells, calcium salts were deposited. Robertson thought that they probably arose from the retention of pus in the tonsillar substance. Walsham considers that some arise from the debris of epithelial pearls in the lining of the crypts.

We believe that they have their origin entirely in the crypts and never in the tonsillar substance proper. Given a condition of retention of debris, which is mostly epithelium, in the crypt, and a further disintegration of this material, the deposit of calcium salts is liable to occur, possibly by the attraction of the fatty acids liberated. On the other hand the mycelial growth, mucus, bacteria and debris frequently form small round cheesy masses which can be expressed from the crypts, but which do not contain calcium salts in visible form. To repeat, we believe that the retention with later decomposition of the debris by bacteria is the most important factor in their formation.

Fibrosis of Tonsil.—Chronic interstitial tonsillitis or fibrosis is an end result of the acute inflammations in the tonsil, and very commonly it is associated with them. As the name indicates, an increase in the amount of fibrous tissue is the principle feature, and hence the term fibrosis is to be preferred to chronic parenchymatous or follicular tonsillitis. As far as we have been able to observe the follicles show no characteristic change, nor do they appear to share in the fibrosis which involves only interstitial tissue about them.

Wright and Hurd believe that this process is an end result of a tuberculous lesion; but it is probable that a pyogenic infection rather than tuberculosis is in the vast majority of cases the primary cause. Nevertheless tertiary syphilis as a possible factor must not be overlooked, although syphilitic fibrosis in view of its infrequency, cannot be regarded as a very likely cause.

In general the connective tissue of the tonsil is derived from the capsule and the trabaculae; the latter probably giving rise to the greater part of the supporting stroma. In fibrosis, therefore, we may meet with one of two conditions,—an increase in the size of the trabeculae or an increase in the amount of stroma in the parenchyma of the organ. These two forms are commonly associated in the same organ. Of fibrosis itself there is nothing of note, save that it confines itself to the site of the normal interstitial tissue, and does not involve the follicles. Where the trabeculae are chiefly affected they become very much enlarged, while from their margins strands of connective tissue spread out into the parenchyma of the tonsil coupled with marked infiltration of lymphoid cells along the lymphatic channels. In some of our cases a considerable increase of perivascular connective tissue was to be seen, especially about the smaller vessels in the parenchyma of the tonsil. This engorgement of the lymphatic vessels of the trabeculae is not only seen in fibrosis but also in the other types of chronic inflammation. Whether there is an increase of lymphoid cells in places other than the lymphatics of the capsule and trabeculae, as in the interfollicular tissue, is very difficult to say. The normal tissue of these parts is already so densely packed with round cells that to differentiate an increase in one or other type is very difficult.

Plasma cells are present and are especially well seen along the trabeculae and capsule, but these cells are also normally present in fair numbers in the parenchyma of the tonsil. Eosinophiles are not usual, and when present are frequently found diffusely scattered in large numbers throughout the tonsil. The exact significance of an infiltration by eosinophile leucocytes is not clear. True it is that as in other tissues, it represents a sub-acute or chronic inflammatory reaction, but just what relation these cells bear to the healing process cannot be indicated.

The relation of the extent of the fibrosis to the gross appearance of the tonsil is often quite misleading. In the small hidden and shrunken looking tonsils fibrosis is naturally expected, and is usually present, but on the other hand we may also have an enormously enlarged tonsil showing an equal increase in fibrous tissue in the stroma or trabeculae. We believe, however, that the tonsil gradually passes from the hypertrophic into the atrophic fibrosed state.

Chronic Peritonsillitis.—Chronic inflammation involving the capsule and pericapsular tissue is by no means uncommon. The inflammation is characterized by an increase in fibrous tissue in the capsule and pericapsular tissues, with an infiltration of the characteristic inflammatory cells.

The capsule may become very much thickened by fibrous tissue and this may also involve the large trabeculae which arise from it. In connection with the development of these large bands we must refer to what is mentioned elsewhere, namely, the formation of new tissues as bone and cartilage in the capsule and its surrounding structures. This process begins with an increase of fibrous tissue, developed secondary to chronic inflammation, and by a process of metaplasia the fibrous tissue is converted into cartilage which in its turn may change to bone. This, however, is only occasionally seen.

The infiltration of lymphoid cells as an evidence of a chronic inflammation is a more uniform finding. Lymphoid and plasma cells are always present, and eosinophiles and mast cells are not infrequently seen. This infiltration extends for a considerable distance into the pericapsular tissue and in the lymphatics between the muscle bundles; but a still more common situation is around the mucous glands and ducts. The ducts particularly show this lymphoid infiltration about them and this has led us to believe that the infection enters the pericapsular tissues through these channels quite independent of the tonsil. Whether all pericapsular inflammations are of this origin we do not say, but we are of the opinion that the majority of them are, and in this class must be considered acute pericapsular tonsillitis (quinsy). This would indicate that the tonsils are by no means the only channels by which infection may enter the system from the mouth, and particularly is this of interest in regard to the development of tuberculous lesions in the glands of the neck. In fact, it is evident that the entrance of bacteria through the mucous ducts lying in the pericapsular tissue is quite as probable as through the tonsil, particularly as the blood supply is less in the former than in the latter, and destructive phagocytosis would be equally less; the bacteria would, therefore, more easily gain access to the cervical lymph glands. In some cases, where the chronic inflammation is marked, the mucous glands have shown mucous and other forms of degeneration.

HYPERTROPHY OF THE TONSIL.

Hypertrophy is one of the most frequent conditions seen in the tonsil. Its etiology has been attributed to a number of factors. By many it is believed heredity plays a very important part in its causation, especially in those children where it is associated with a ''scrofulous dithesis.'' This has been clearly shown by Birkett. In fifty-three families he has noted that adenoids of the pharynx occurred in at least two or three children. In those cases of enlargement of the tonsil occurring in infants, causes, other than heredity, are difficult to prove. It is well to remember, however, that the lymphoid structures in infants are always relatively larger than in adults.

The other points in the etiology of hypertrophied tonsils may be divided into constitutional and local causes. The constitutional or predisposing causes are the presence of tuberculosis, syphilis, gout, and alcoholism in the parents, while the importance of acute infections has been mentioned by some observers but denied by others. Hertoghe believed that in myxoedema a general enlargement of the adenoid tissue in the neck was present. This has not been verified. Osler considers that the tonsillar hypertrophy is associated with a disturbance of the general glandular system which may be of the type of lymphaticism or status lymphaticus. Ewing regards any lowered vital resistance from inherited or acquired influence a factor in enlargement of the tonsil, and he believes that rickets, anemia, hemophila, disturbance of the thyroid and genital hypoplasia, are the more common causes. Local causes, as recurrent attacks of tonsillitis, are by many believed to be of only minor importance. From our observations we are by no means convinced that inflammation plays the lesser role in the production of hypertrophy, in fact, from our studies we have been led to take quite the opposite view, and regard tonsillitis the most important factor in the production of the enlarged or hypertrophied tonsil. That constitutional causes play a part in the process of hypertrophy, and that there is a relationship between the constitutional and the local cause we believe is quite definite, but it is also evident that the local causes—recurrent pyogenic inflammations—are the more important. Obviously recurrent attacks of tonsillitis are of more significance than a single attack. In studying a long series of cases we find that some recurrent inflammations lead not to enlarged, but to contracted and fibrosed tonsils. This we have referred to in another place, as a later stage of a preceeding hypertrophy. If the hypertrophy is of long standing a secondary contraction due to fibrosis is the probable outcome. McBride and Turner have referred to this in connection with adenoids, but Nicholl and Lartigau point out that atrophy and fibrosis do not always have a relation to one another.

The gross appearance of the hypertrophied tonsil shows nothing of note save that it is enlarged. The tonsil is soft, and the mucosal surface is convex. This bulging of the surface may become quite prominent, so that the mouths of the crypts are compressed and narrowed. If there is a considerable lacunar inflammation in the deeper portions of the crypts the openings will be wide and gaping, and exudate may be seen at their mouths.

The normal tonsil measures 2.5x1.8x1.5 cm., but this measurement is not constant as the age and size of the individual have some influence upon it. Tonsils larger than this are classed as hypertrophied. There are fairly wide variations in size of hypertrophied tonsils, our largest measuring 4.5x4x3 cm. The tonsil tends to enlarge up to puberty, and then contracts; but inflammatory conditions may entirely alter the structure so that we may have a maximum size either in childhood or early adult life. There is also a relation between the size of the individual and that of the tonsil, as larger children have relatively larger tonsils. Usually the only distinctive point, clinically, in the gross appearance of the tonsil is its actual size. It is therefore not surprising that the usual clinical diagnosis which sends the child to the specialist for removal of tonsils, is ''enlarged'' or ''hypertrophied'' tonsils, without any reference to the actual pathological condition in the organ.

There is no characteristic or uniform microscopical picture of hypertrophy of the tonsil. Nevertheless, when properly looked for, there is practically always some form of inflammation present, the acute and chronic lacunar tonsillitis being the most common. The enlargement of the tonsil is due to an increase, both in number and size, of the tissues and structures of the part. It has been pointed cut by many observers that in hypertrophy the follicles are not only larger than the normal but are also more numerous. We do not believe that the numerical determination of the follicles in one or more sections of a tonsil can be of any value in determining hypertrophy of the organ. That almost all tonsils vary in the number and size of follicles present in sections is easily shown by the examination of a number of slides; but to estimate whether there is hypertrophy of the tonsil from these facts, we consider to be quite unreliable and often misleading. We had hoped that the presence of nuclear figures indicating proliferative changes in the cells of the follicles would be of value in determining the presence of hypertrophy, as it was thought their number would probably be increased in the rapidly growing follicles. Our findings, however, were not conclusive enough to be of practical aid. Some of the smallest tonsils with inflammation showed more of these nuclear figures than the large ones, the latter of which were examples of clinical hypertrophy.

Increase in the stroma has also been spoken of as a factor in hypertrophy, and undoubtedly it is seen in some cases. On the other hand it was found that there were just as many hypertrophied tonsils which did not show it. In fact quite the opposite result was obtained in the small contracted tonsil with fibrosis, where it was noted that the small hard tonsil had a very dense stroma and small follicles. There was no special change as far as we have been able to make out in the lymphoid cells which make up the greater part of the interfollicular tissue, nor was there any evidence of change in the circulation as distinguished by an increase of the capillaries.

Davis found that in hypertrophy of the tonsil there was a very marked increase in plasma cells, which was roughly proportional to the size of the tonsil. He regards the plasma cells in the tonsil as an indication of chronic infection, and found that these cells were present in greater or less quantity in all individuals after the second or third week of life. We have noticed more particularly in chronic peritonsillitis that plasma cells were present in large numbers about the areas of reaction, while in chronic lacunar tonsillitis and fibrosis of the tonsil, the number of these cells did not appear to vary much from the normal tonsil. That the plasma cell is a constant finding in enlarged tonsils is due to the fact that hypertrophy has always an inflammatory basis; but we are not convinced that the number of plasma cells and the grade of hypertrophy have any relation to each other.

If the question of hypertrophy of the tonsil is viewed from the standpoint of its pathological conditions, it is found to indicate a past or present process of inflammation. And further it is evident from what we have said respecting acute inflammation, that the lesions likely to be associated with a general systematic infection is the acute lacunar type which we find always possesses one or more ulcers. Hence, too, those conditions of hypertrophy of the tonsil, which have an association with or are caused by acute lacunar tonsillitis, are the most frequent types having a direct relation to systematic infections. It is probable that those hypertrophied tonsils, which are the result of a previous inflammation but which have subsequently healed, are less frequently associated with infections beginning in the mouth. Such perfectly healed conditions, however, in tonsils which are yet hypertrophied, are rare.

Therefore, although enlargement of the tonsil is due, in all probability, to hypertrophy and hyperplasia of the cells of the part and particularly of the cells in the center of the follicle, there is no microscopic picture which is characteristic of it alone. We would repeat, that the enlargement of the greater majority of the so-called hypertrophied tonsils, both in adults and children, has an inflammatory basis and must be treated as such.

MYCELIAL INFECTION OF THE TONSIL.

The presence of a variety of mycelial forms is not uncommon in the buccal cavity, and likewise a similar variety of moulds and higher bacteria have been observed in and about the tonsils. In 1881, Johne reported the findings of a streptothrix in the tonsillar crypts which he believed was actinomyces. Volkman some years later, as well as Wright, reported a similar finding in the crypts, while the former also noted the same infection in and about decayed teeth. Bauermeiser observed this organism in the crypt of the tonsil in hogs. These observers all laid stress on the fact that the tonsil itself was otherwise healthy. Lord, within the last two years, made similar observations both upon the crypts of the tonsil and upon decayed teeth. Further, out of sixty per cent. of his cases he was able to produce lesions simulating actinomycosis in guinea pigs by intra-peritoneal innoculation.

In our series we have been able to demonstrate a mycelial growth in the crypts in about twenty-three per cent. of the cases. We feel, however, that this number errs on the small side and that if more portions were taken from each tonsil for section, these structures would be found much more frequently.

The mycelial structures were localized entirely to the crypts and were never found in the tonsillar substance proper. In structure they were quite similar to colonies of actinomyces except that their clubbed extremities were not so large nor so regular, but they were almost always present. The filaments could be stained by Gram's method quite deeply while the clubs were only faintly stained but still were not completely gram negative. They were also slightly acid fast. The colonies usually were in association with numerous other bacteria which were present in the crypts but they did not appear to cause any special outpouring of leucocytes around them. In fact at times they were found lying free in the crypt without the slightest evidence of polynuclear infiltration or other inflammatory reaction.

The question immediately arises whether we are dealing with the same organism that has been previously described, and is it the pathogenic actinomyces? In all probability the organism is the same as was noted by former observers, but we do not regard it identical with actinomyces bovis or hominis. Lord believed that the presence of so many other organisms in the crypts hindered its growth and hence it rarely produced true actinomycotic lesions. However, in view of the fact that he produced typical granulomatous lesions in the guinea pig, he concluded that it was actinomyces.

When one considers how infrequent true actinomycosis of the tonsils is and how frequent fungi of this order are found in the crypts, it is strong evidence that we are not dealing with a latent form of this infection. We have observed this fungus present in tonsils which were otherwise the seat of ulcerations within the crypts and yet there was no attempt on the part of this organism to invade the tissues.

These moulds must be considered as non-pathogenic forms (a type of streptothrix) and are not the same as those which produce actinomycosis. There are several types of harmless streptothrices present in the air and it is probable that these found in the tonsil are of this nature. What etiological relation these organisms may bear to any of the inflammation conditions in the tonsil is difficult to say. We know that in those cases of recurrent attacks of tonsillitis, not infrequently small yellow plugs of a foul smelling material can be expressed from the crypts, and that in these plugs almost invariably one finds a filmaentious growth which has the same structure as those seen in sections of the tonsil. Numerous other bacteria are of course always present, so it is an uncertain matter at the present time to fully realize their significance.

TUBERCULOSIS OF THE TONSIL.

That the tonsil is affected by a tuberculous process has been known for a long time. Virchow was one of the first to describe this condition, and on account of its relative infrequency, he believed that the tonsil had an unusual immunity for tuberculosis. Since that time a large number of cases have been reported, and consequently the condition is known to be much more usual than was formally supposed.

Tuberculosis may involve the tonsil as a primary or a secondary infection. According to the collected cases in the literature, by far the great majority of the lesions are secondary to another infection of the respiratory tract. Some bold statistics have been advanced by various observers; one to indicate the frequency of tuberculosis as a primary invader of the body through the tonsil, the other to illustrate how commonly the tonsil becomes infected when sputum laden with tubercle bacilli is in contact with the organ.

Schlesinger found among thirteen cases of active tuberculosis of the lung, twelve with tuberculosis of the tonsil. In four other cases of obsolescent pulmonary tuberculosis he was unable to demonstrate any tonsillar involvement. He concluded that active pulmonary tuberculosis was commonly associated with tuberculosis of the tonsil, and conversely that tuberculosis of the tonsil was definite evidence of pulmonary involvement.

Strassmann found twenty-four cases of tuberculosis of the tonsil in those suffering with well marked pulmonary tuberculosis. Schlenker believed it occurred only in individuals having recent and progressive pulmonary tuberculosis. He failed to observe it in a child of ten and a half months, who was suffering from a caseous tuberculosis of the lungs. This apparent discrepency was due to the fact that in children there is a very little sputum and hence few bacilli are washed over the tonsil. Dmochowski found fifteen tonsillar infections in as many cases of pulmonary tuberculosis. Macroscopically, he states, tuberculous lesions were not visible, but were revealed only with the microscope. Kruchmann reported twelve cases in twenty-five autopsies on tuberculous patients, while Walsham found twenty out of thirty-four who were suffering from pulmonary phthisis. Wood collected a large number of cases of undoubted secondary tuberculous tonsils, and found that sixty-nine per cent. of the cases with pulmonary involvement showed also tonsillar infection. Only in patients with active lesions, however, was this true, as in all cases the sputum contained bacilli. In healed tuberculosis of the lung the disease was not found in the tonsil. Ruge had six cases of tonsil infection in eighteen individuals examined, and Pluder found tubercles in five out of thirty-two. White reported one tonsil involvement in seventy-five cases. Wex found seven cases in two hundred enlarged tonsils. Chiari in a study of the tonsils of six hundred individuals, found evidence of tuberculosis in 1.88 per cent. Levy in a similar analysis of four hundred and fifty individuals with tuberculosis of the respiratory tract found tuberculous lesions in the tonsil in 1.77 per cent.

Out of three hundred and fifty pairs of tonsils in our series in which lesions of tuberculosis were searched for, we found five cases with tubercles in the tonsils, or 1.4 per cent. of all cases.

Of the primary involvement fewer cases are reported and many of them, too, are somewhat doubtful. An undoubted case of primary tuberculosis of the tonsil can only b erecognized by autopsy examination.

Ruge reported a case of food infection of the tonsil in a girl of nineteen with secondary development of tuberculosis in vertebra. The case is not without some doubt, whether obsolescent tuberculosis was present in or about the lungs. Gottstein reports a case of bilateral primary infection. A secondary infection, however, is not entirely ruled out. Kruckman believed that five out of a total of twenty-five cases examined by him were primary. Wood has collected from the literature eighty-eight cases of primary tonsillar tuberculosis from sixteen hundred and seventy-one cases studied, while Robertson reports eight per cent. of primary tuberculosis in two hundred and thirty-two cases examined.

Thus in summing up the literature we find there is a wide variation in the results. The opinion, however, that primary tuberculosis of the tonsil is rare, and that secondary infection is the one usually seen, is now fairly general. The great frequency noted in some reports of secondary infection of the tonsil is probably to be accounted for in the type of patients under observation. Thus in many instances the frequency of the infection was based on the examination of the tonsils taken from cases suffering from acute tuberculosis, which obviously had repeated sputum contamination with tubercle bacilli. In healed tuberculosis of the lung the tonsils were as a rule not found to be involved, and it has been advanced by some that in these cases the lesions in the tonsil had disappeared, or as Wright and Hurd believed, was represented by a fibrosis of the organ. Of the former contention it does not appear at all probable, nor even possible, that a definite tuberculous lesion in the tonsil could entirely disappear without leaving some evidence; on the other hand, as we have referred to elsewhere, we cannot regard the presence of fibrosis as alone indicative of tuberculosis. Another possibility, which we believe to be more probable, is that the obsolete pulmonary lesion described by some, was originally a type of miliary or localized infection, and that the sputum, therefore, never contained tubercle bacilli, consequently the tonsil was not involved. In the cases reported by Wex, Chiari, and myself, the tonsils had been removed merely for hypertrophy or recurrent attacks of tonsillitis, and from clinical evidence none of my cases had active tuberculosis in any form.

In our cases the ages were 2, 11, 12, 18 and 21 years respectively. Active pulmonary tuberculosis was not made out in any case, but still we feel that we cannot even suggest that the tonsil infection was primary, in view of the fact, that there might have been a small pulmonary infection, which could not be recognized by physical examination. It is a very uncertain matter to indicate that any tuberculosis process is the primary site of infection unless the findings are based upon a thorough postmortem examination.

The tuberculous lesions in the tonsil may consist of superficial ulceration, small miliary tubercles or large caseating nodules.

Walsham has pointed out the frequency with which tuberculosis has been entirely overlooked in the gross specimen but was readily recognized on microscopical examination. He, therefore, gave the name of latent tuberculosis to this form. Although all of our cases were of this type, still we are not in sympathy with the term, and believe that in some cases, a thorough inspection would reveal tuberculosis. Moreover, by his use of the term ''latent'', there is no indication of the activity of the disease process,—a point which is much more important. In our cases the presence of typical tubercles on microscopic examination was considered sufficient to diagonse tuberculosis. In all we are able to show caseation necrosis, endothelial cells, epitheloid cells, connective tissue change, and giant cells. We have, however, been unsuccessful in demonstrating tubercle bacilli in each of the sections. We encounter only one other condition which might lead to any confusion or be mistaken for a tuberculous lesion, and this is the isolated foreign body giant cell, which is occasionally seen near the margins of the crypts. In absence of other changes in the tissues these were quite readily distinguished from the giant cells of true tubercles. Necrotic areas in the tonsil, other than those of acute pyogenic infections, we have not observed.

By what route are the tubercle bacilli carried to and into the tonsil? Here, necessarily, we must consider two types of infection, primary and secondary tuberculosis. In primary tuberculosis the infection comes into the mouth by food or in the inspired air. Gottstein believed that fine particles of dust laden with bacilli fall on the mucous surfaces of the tonsil and from here the tubercle bacilli work their way into the substance of the tonsil. Therefore, he contends that in cases of enlarged tonsils, the surface area of the organ being greater, the incidence of tuberculous infection also increases. However, other observers take quite another view, holding that enlargement of the tonsil has no relation to tuberculosis. From the analysis of our cases it would appear that hypertrophy does not tend to increase the frequency of tuberculosis of the tonsil. In fact, tuberculosis probably occurs more frequently in the so-called hidden or contracted tonsil than it does in the enlarged one. Three of our cases were small tonsils. Ruge accounts for the primary tuberculous infection in a slightly different way. The infection, he holds, whether it be from food or air, is brought to the tonsil by the sputum. This is most probably the case. Thus the infected air or food deposits tubercle bacilli upon the moist surfaces of the mouth and pharynx, and even when the organisms are not deposited directly upon the tonsils they are not infrequently washed over the surface of these organs and lodged in the mouths of the crypts.

In secondary tuberculosis the infection is carried to the tonsil by the sputum or blood from some other focus in the body, while Ruge contends that the lymphatics may also be a portal of entry. The concensus of opinion is, that in the vast majority of cases the bacilli are carried by the sputum from an infection in another part of the respiratory tract, most commonly the lungs and the larynx. Stohr believed that bacilli were carried into the tonsil by the leucocytes through the ''physiological wounds'' which we have previously described. A number of observers, including Rindfleisch, Treip, and Gerhardt have supported this view. Dmochowski also agreed with this, but added that the bacilli may enter directly at previously ulcerated surfaces. Wright and Hurd and White maintain that the bacilli pass directly through the epithelial lining of the crypts. It does not appear likely that the infection enters by "Stöhr's physiological wounds", but it is more probable that the infection extends through an ulcerated area in the epithelial lining of the crypts directly into the tonsil substance, and seldom if ever passes through an intact epithelial layer. We, therefore, accept Dmochowski's contention.

As we have previously pointed out the majority of inflammatory conditions of the tonsil show collections of polymorphonuclear leucocytes in the crypts, and we believe that these leucocytes are derived from ulcers involving the epithelial lining. The ulcers are due to some pyogenic infection and never show primary evidence of tuberculosis. Here then, in acute lacunar ulcerations, we have a factor which is a predisposing cause for a tuberculous invasion. Tusseau has previously pointed out that chronic inflammation of the tonsil is a predisposing factor, and with this we agree, remembering, however, the frequency with which acute lacunar ulcerations occur in a recurrent or chronic tonsillitis.

Secondary tuberculous infection carried to the tonsil by the blood stream is quite possible and needs no comment. It is only necessary to state that this route is an infrequent one and confined to those cases of extensive miliary distribution in which the whole body is flooded by infection. Infection carried by the lymphatics, as an ascending infection, is supported by Ruge. The majority of observers believe it to be extremely rare if it occurs at all.

Experimentally, Wood, Baup and Ravenel have produced tuberculosis of the tonsil in hogs and rabbits by swabbing the pharynx and tonsils with a solution of tubercle bacilli. General infection is reported to have followed in these cases. From these observations it was concluded that primary tuberculosis in the tonsil was possible. and that dissemination of the disease to other parts of the body could occur from it.

The relation of the tonsil to tuberculosis of the glands of the neck is of great interest. Many observers have reported that the tonsils are the portals of entry for these tuberculous lesions, and some believe that even the lungs are secondarily involved by a descending infection through the chain of the glands in the neck. Robertson states that he has frequently noted this route of infection. Schlenker described ten cases of bilateral tuberculous tonsillitis, among whom nine were cases of tuberculous cervical adenitis. In them he showed that the infection was of the descending type. However, the greater number of cases of tuberculous cervical adenitis are without tonsillar involvement, and Schlenker who believes the tonsils were at fault explains this in one of three ways; that, (1) The organisms have entered by some other portal of entry, (2) the invasion into and through the tonsil has occurred without visible lesions, or (3) a tuberculous lesion of the tonsil has healed so that it no longer can be recognized.

Kruckman has made observations and come to conclusions similar to those of Schlenker. Wood believes that ninety per cent of the tuberculous cervical adenitis are secondary to tonsil infection. On the other hand Watson Cheyne, considering the infrequency of tuberculosis of the tonsils as compared with that in the cervical glands, was strongly opposed to the view that they acted as channels of infection in tuberculous adenitis of the neck. He referred to the work of Delphine and Younge to substantiate his views. These observers found that in seventeen cases of tuberculous cervical adenitis the tonsils were quite free from these lesions. Cheyne also brought out the point, that if the tonsils so readily absorbed the bacteria why should we not get tuberculous cervical adenitis more frequently in acute pulmonary phthisis. Walsham, however, had a very different experience. In his autopsies on twenty-seven cases of pulmonary tuberculosis he was able to demonstrate, that the cervical glands were involved by a tuberculous process in twenty-six of that number, the one exception being a case of fibroid phthisis. He points out that negative findings have been made mostly on clinical and macroscopic appearance of the glands. Moore has interpreted the findings of Delphine and Younge in a different manner to Watson Cheyne. He considered that the failure to find tubercles in the tonsil was due to the fact that the tonsils though serving as a route of infection, had in itself not reacted to the infection.

It is to be repeated that statistics concerning the relationship of different tuberculous processes, and the determination of the primary infection and the route of distribution can only be obtained by careful autopsy examinations. In our post-mortem analyses we have been particularly impressed by the frequency with which the lymph glands at the bifurcation of the trachea show the oldest lesion, while the more recent, active and caseous processes are found along the border of the trachea higher up. We have never seen a case in which a chain of tuberculous glands from the neck to the peribronchial system showed the oldest lesion above. This is also in agreement with the findings of Ribbert.

We have frequently been unable to demonstrate tuberculous lesions in the tonsils in cases which had or previously had had caseous cervical glands removed, and moreover, our cases (five) having tubercluosis of the tonsil showed no evidence of tuberculous adenitis of the neck. It must be remembered that infections to all parts of our body may be received through other portals than through the mouth.

From our observations on pericapsular tonsillitis, which have been described elsewhere, we believe that the pyogenic infection may also enter through the mucous ducts of the pharynx quite independently of the tonsil, and there is no reason to doubt that tubercle bacilli may enter by the same route. Whether tubercle bacilli can pass through the tonsil without leaving any lesions is a very difficult question to answer, and it also leads to the discussion of the statement, first made by Virchow, that the tonsil has some immunity to tuberculous infection. Walsham and Brieger believed that the tonsil had a strong bactericidal action, due to the out-pouring of polymorphonuclear leucocytes. That the phagocytic action of the polymorphonuclear leucocytes is well marked in the tonsil is not to be denied, but we also know that in active pulmonary phthysis the percentage of involved tonsils is high, so that it does not appear that the tonsil possesses any special immunity for tuberculosis.

We have learned through the experience of Cornil, that the occasional finding of tubercle bacilli on the surface of or in the crypts of the tonsil, in the absence of tubercles in the substance of the organ, accounts in part at least for the discrepancy of the statistics on the frequency of tuberculosis of the tonsil. By some authors the animal test alone was used. The macerated tonsil was inocculated into guinea pigs and the positive or negative results in these animals was held to indicate the presence or absence of tuberculosis of the tonsil. It is clear that these results are fallacious, and that the frequency of true tuberculous lesions is overestimated, in that the positive guinea pig results also include the infections obtained from tubercle bacilli adhering to the surface without being associated with any lesion within the organ.

White, however, was unable to produce tuberculous lesions in guinea pigs from materials inocculated from tuberculosis tonsils. This he considered was due to either a diminished virulence or else to the unequal dissemination of the lesions which had only involved a small part of the tonsil, so that possibly the tissue injected did not contain any tuberculous material.

There are three outstanding points respecting tuberculosis of the tonsil; (1) Statistics on the frequency of primary and secondary tuberculosis of the tonsil are very misleading and difficult of comparison, unless each is accompanied by proof of a thorough physical examination indicating the presence or absence of tuberculosis in other parts of the body; (2) It is questionable whether the diagnosis of primary tuberculosis of the tonsil can be made with accuracy during life; (3) The frequency of tuberculosis in hypertrophied tonsils obtained from apparently healthy individuals, is low, varying from one to two per cent.

SYPHILIS OF THE TONSIL.

In the not infrequent syphilitic lesion of the mouth and pharynx the tonsil is very liable to be the site of the infection. In primary extra-genital lesions, the tonsil is most usually involved, due in all probability to the fact that its anatomical structure gives greater opportunity for the spirochaeta pallida to locate, than is elsewhere afforded in the mouth or pharynx. The same factors govern the frequency of acute pyogenic infections of the mouth. Syphilis involving the tonsil may be either primary, secondary or tertiary.

Primary syphilis (chancre) is rare and when occurring in the mouth or pharynx is usually situated on the tonsil. The first cases were reported by Ricord in 1838. Roun in 1852 reported 82 cases, and since then a large number have been brought forward from time to time, so it is probable that the condition is not as rare as we would suppose.

The data from the literature indicate that in the extra-genital primary sore the involvement of the tonsil ranges from 6 to 55 per cent. Pospelm had forty-six cases out of 198 cases of extra genital lesions, Helling 22 in 101, and Newman found 6 per cent were localized to the tonsil. Women are more frequently affected than men, and the right tonsil was involved more than the left. The lesion, occupying as it does, the greater part of the mucus surface of the tonsil, shows a broken appearance with fairly sharply defined margins having a considerable amount of induration around it. The surface is moist and a dirty grayish secretion is present on it. Typical of syphilitic lesions there is usually very little pain, although in exceptions, severe pain may sometimes be present. The lymphatic glands in the neck and those situated under the angle of the jaw are enlarged and tender, but never undergo suppuration.

Secondary syphilis (metastatic) first shows itself in the mouth and pharynx by a slight redness which involves the soft palate and fauces, but which is supposed very rarely to enter the tonsil. However, some recent observations contradict the statement. Campbell has shown that the spirochaeta pallida can be isolated from the tonsil by scraping the mucous surface after it has been cleansed of its debris with a fine currette, and can be demonstrated in the exuded serum by the dark stage illumination. He was able to find the spirochaeta pallida in syphilitics who revealed no other evidence of the disease in the mouth save a slight congestion of the tonsil. He concluded that the spirochaeta pallida appears in the tonsil shortly after the initial lesion on the penis, and some time previous to the earliest evidence of mucous patches.

Mucous patches more commonly involve the soft palate and the lateral lining of the mouth than the tonsil. When closely observed, however, the tonsils are as a rule found to be involved with the rest of the mouth. The mucous patches appear about the fourth month. Another type of lesion noted in the early secondary stage about the time of the skin eruption, is the superficial ulceration of the tonsil. These ulcers are symmetrical and kidney shaped having a grayish base and sharply defined margins. They do not tend to spread laterally or extend into the tonsil. As a rule they are small and not painful. Jonathan Hutchison considers that they are rarely absent but can be easily overlooked when they are minute. They heal slowly, often only after some weeks.

Tertiary syphilis (primary or metastatic in origin) is seen in the form of a gumma or an ulceration which has developed by the superficial erosion of a gumma. Tertiary syphilis of the tonsil is not common. The gumma appears as an irregular red swelling covered by a smooth congested mucous membrane. It is not painful and causes discomfort only by its size, especially in swallowing. The central part soon becomes necrotic on the surface. In this manner tertiary ulcers are formed. The ulcerations, therefore, are deep with irregular grayish-yellow and sharply punched out edges or margins. At times severe haemorrhage may occur when the ulceration has eroded a vessel. The whole tonsil may be involved in the ulceration. Following the healing of the ulcer there is a great deal of scar tissue formed, due to the replacement of the loss of a considerable substance of the organ in the early process. This then develops a very puckered, contracted and fibrosed tonsil showing stellate scars on its surface.

BONE AND CARTILAGE IN TONSIL.

Small islands of bone and cartilage in the tonsil have been seen and reported upon by a number of observers. These islands are small, occurring only in the connective tissue of the capsule and trabeculae, and are to be observed only microscopically. Deichert has reported two cases, one occurring in a man of fifty-nine years, dying from a fractured skull, and the other in an infant. In the infant, however, only cartilage was found. Orth and Toepfer have commented upon similar changes. Walsham found five cases in a total of two hundred and thirty-four. All of his cases occurred in adults of about twenty-two years of age, and the abnormal tissue was always found in the capsule or the trabeculae. To account for these structures several theories have been advanecd.

Deichert believed that the occurrence of cartilage in the tonsil was due to an anomaly of development in the bracnhial clefts. In the process of development of the tonsil, arising as it does from the branchial arch which contains cartilage, it was thought that a small portion of this cartilage might be torn off or implanted into the tonsil. He also suggested that the eustachian tube or stylohyoid ligament, which contains cartliage, might also be the point of origin. It was inferred by some that possibly we had to do with cell rests of the nature of Cohnheim's theory but Deichert points out that the symmetrical arrangement and location does not suggest a new growth from cell rests. Deichert thought that an abnormally long styloid process of the temporal bone, which always had a cartilaginous end, might by its proximity implant some of this tissue into the tonsil. Walsham first believed that the presence of cartilage and bone was of the nature of a new growth, but in view of the findings of Deichert, he concluded that it must be of foetal origin.

Toepfer points out that in order to get at the root of the matter we must consider that the bone is merely developed from the cartilage and we need, therefore, seek the origin of the cartilage.

Discussing Deichert's theories he points out that since the development of the tonsil begins between the fourth and fifth month of foetal life, the origin of cartilage from these structures is not probable, as the branchial arches have long since disappeared. One thing that appeared to impress itself upon the author was that the development of cartliage was in association with the heavy bands of connective tissue. In his case he felt quite sure that the connective tissue cells entered directly into the cartilaginous areas and he inferred that the cartilage cells arose from the connective tissue cells. He speaks of this as a metaplasia and believes that we may refer to it as an enchondroma as it arises from no performed cartilaginous tissue.

From our own observations we draw the same conclusions as Toepfer, and consider that the process as one of metaplasia and not a developmental error. We have collected eleven cases in three hundred and fifty pairs of tonsils. Four had cartilage present alone, one had bone alone, and six had both bone and cartilage. These abnormal structures were always situated in the capsule or large trabeculae and never extended into the tonsillar substance proper. They were recognized only by microscopic examination, and the large majority of them occurred in small fibrosed tonsils. As regards the ages of occurrence we have had some interesting data. In the four cases where cartilage alone was present the ages were 4, 6, 8, and 21 years, an average of 9.75. In those cases showing bone the ages were 14, 12, 22, 31, 37 and an adult age not known, and average of 23.5. Thus from our own cases, and the same holds true from those of others, it is found that cartilage alone is seen in the tonsil at a much earlier age than bone, while bone appears only in late youth or early adult life. The development of bone is thus a matter of some years. This we believe is another point in favor of metaplasia as a theory of its development. Seven were in females and four in males.

The microscopic characters of the tonsil presented a constant feature in the presence of very heavy connective tissue bands around them. The connective tissue cells were very closely attached to the cartilagenous areas and appeared to be directly associated with them, apparently entering into the formation of the typical cartilage cells. In view of the fact that nearly all of our cases occurred in small fibrosed tonsils, which we regard as the end result of chronic inflammation, we therefore feel that inflammation of the chronic type is a predisposing factor to the development of aberrant cartilage and bone.

We encountered an interesting feature in two of these cases, the association of tuberculosis with these aberrant tissues in the tonsil substance. It would seem possible that the tuberculous process served as a predisposing factor for these tissues in bringing about a fibros is of the organ. As far as our own observations show, bone and cartilage in the tonsil is of very little importance, save as an unusual finding or for the study of metaplasia.

ASSOCIATED CONDITIONS.

Other than the immediate effects and changes occurring in the tonsil as the result of inflammatory processes there are a number of conditions which are more or less distantly related to inflammation that we must briefly consider. When taken individually some of these associated conditions would appear to bear no relation to the main subject under discussion. But on a closer scrutiny, an interrelationship or even a dependence of one upon the other is observed.

It would be unwise to discuss the malignant tumors in connection with the subject in hand. And moreover, the lack of a sufficient personal experience with primary malignant tumors arising in the tonsils, forces me to decline any discussion upon them. On the other hand, we have had an opportunity of studying a number of benign tumor masses of the tonsil, some of which appeared to have a sufficiently suggestive relation to certain inflammatory states to bring them into the present discussion. Particularly in the case of two benign polypi did we believe that there was definite evidence of a chronic inflammatory process preceding the tumor mass.

Papilloma of the tonsil is the most frequent benign tumor. It is a warty, cauliflower growth situated on the mucous surface of the tonsil at or near the margin of the opening of the crypt. These growths vary a great deal in size but are usually quite small. They are not painful or congested. One such growth studied was situated near the lower border of the tonsil on the mucous surface at the margin of a gaping crypt. The growth measured 0.5 cm. in diameter (transverse) and 3 m.m. in thickness. Its surface was typically warty. It was made up of stratified squamous epithelial cells with a thin keratinized layer on its surface. There was no evidence of encroachment of the new growth into the tonsillar tissue. Epithelial pearls were not present. Under the epithelial structure, a lymphoid cell infiltration without definite arrangement was seen. There was one point of interest in the outline of the growth. Situated as it was, on the margin of a crypt, it extended downwards for a considerable distance into the tonsil along the lining of a crypt, giving rise to an appearance almost identical with the papillomatous arrangement which occurs in well marked chronic lacunar tonsillitis. The similarity of these two conditions was striking. Papillomata, therefore, may extend beyond the buccal mucous surface into the deeper portions of the crypt.

Polypi are soft, pedunculated growths projecting from the mucosal surface. Their surfaces are smooth and usually congested. They have a thin stratified squamous epithelial covering and the stroma is made up of a delicate fibrous or myxomatous tissue which is well supplied with blood channels. Our second tumor was of this type. It was 1.5 cm. in length and at its widest point 0.5 in diameter. Its pedicle was quite narrow and firm and attached to the mucous surface of the tonsil. The outer surface of the polyp was smooth. The microscopical section showed it to have a stratified squamous epithelial covering and its framework was a loose fibrous tissue in which many large blood sinuses were seen. A number of lymphoid cells were scattered throughout the stroma. The pedicle was continuous with the fibrous stroma of the tonsil.

Our third tumor was a polyp attached to the mucous surface of the tonsil by a fine pedicle. The polyp was globular measuring 1 cm. in diameter. Its surface was quite smooth. The polyp was soft and on cross section the structure had the appearance of a cyst. The wall was thin and the central portion was filled with a yellowish sebaceous-like material, similar to that seen in the dilated crypts containing cholesterin.

Microscopically the wall consisted of an outer layer of stratified squamous cells with some true tonsillar tissue immediately underneath. The inner lining of the cyst was also a stratified squamous layer but it was very thin and compressed. Here and there a few keratinized cells were seen attached to the inner lining and some had desquamated into the central cystic portion. A homogeneous pink material containing some keratinized epithelial cells and a number of polymorphonuclears made up the central area. The picture here is quite similar to the retention cysts of the tonsillar crypts described previously. The pedicle was fibrous and contained many lymphoid cells. Lying between the large cyst and the pedicle a smaller dilated crypt showing marked keratinization of its lining cells, was also seen.

We have then a polyp of quite different characters than the previous one; in fact it would probably be more correct to refer to it as a retention cyst, the assumption of the polypoid shape being due to mechanical measures. It is likely that the structure began as the result of an obstruction to the mouth of a crypt, and with the constant desquamation of cells into it, a marked enlargement resulted. Its situation in the tonsil was immediately under the buccal mucous membrane, and as it enlarged it extended into the buccal cavity, drawing with it a pedicle of the tonsillar stroma. The acute inflammation present is the exception in retention cysts, but in this case the prominent position of the polyp in the mouth would render infection more liable to occur. Ulceration, however, was not present in any parts of the wall.

SUMMARY.

The function of the faucial tonsil does not appear to bear any important relation to the welfare of the body at large. It is evident that the tonsils do not absorb foreign material from the mouth but by means of the 'physiological wounds' they emit varying numbers of lymphocytes and leucocytes. It is possible that these cells may have some bactericidal action in the vicinity of their extrusion.

Acute inflammation of the tonsil begins practically always in the crypt and is characterized by an ulcer in the lining epithelium. In its origin it bears a close relation to the process of acute inflammation in the appendix as described by Aschoff. Acute lacunar ulceration is a frequent finding in those suffering from recurrent attacks of tonsillitis, even during the interval between the attacks.

Hypertrophy of the tonsil has an inflammatory basis, and the constitutional causes are of secondary importance. The microscopic picture is that of an acute or sub-acute inflammation in the crypts differing in no way from other inflammatory states in the tonsil.

The process of metaplasia, as indicated by the presence of bone and cartilage in the capsule of the tonsil, is not infrequent.

The incidence of a tuberculous process in the tonsil, in cases where pulmonary phthysi sis not a factor, is small, occurring in 1.4 per cent of our cases.

The tonsil is by no means the only portal of entry of infection from the mouth. Infection may also enter through the mucous ducts surrounding the tonsil as well as through the other lymphoid structures constituting the lymphoid ring of the pharynx.

(The author wishes to express his thanks to Dr. Oskar Klotz for his valuable assistance in the progress of this work, and to Dr. S. R. Haythorn for the photomicrographs.)

DESCRIPTION OF PLATES.

Fig. 1. Bone and cartilage in the capsule of the tonsil. Lower right corner shows muscle bundles.

Fig. 2. Fibrin in the central part of the follicle following acute follicular tonsillitis.

Fig. 3. Mycelial growth in the crypts.

Fig. 4. Mycelial growth under high power showing extremities.

Fig. 5. Fibrinous exudate in the crypt in acute lacunar tonsillitis.

Fig. 6. Ulceration of the lining epithelium of the crypt.

Fig. 7. Chronic peritonsillitis with infiltration of lymphoid and plasma cells about the mucous glands.

Fig. 8. Tuberculosis of the tonsil.

Fig. 9. A muscle bundle in the parenchyma of the tonsil.

Fig. 10. Dilated crypts containing desquamated kertinized epithelium and cholesterin.

Fig. 11. Keratinization of the desquamated epithelial cells in the smaller branches of the main crypt.

Note the number of branches. This is a feature of chronic lacunar tonsillitis.

Fig. 12. A fibro-myxomatous polyp of the tonsil attached to the surface.

Fig. 13. Polyp of tonsil with central dilated crypt.

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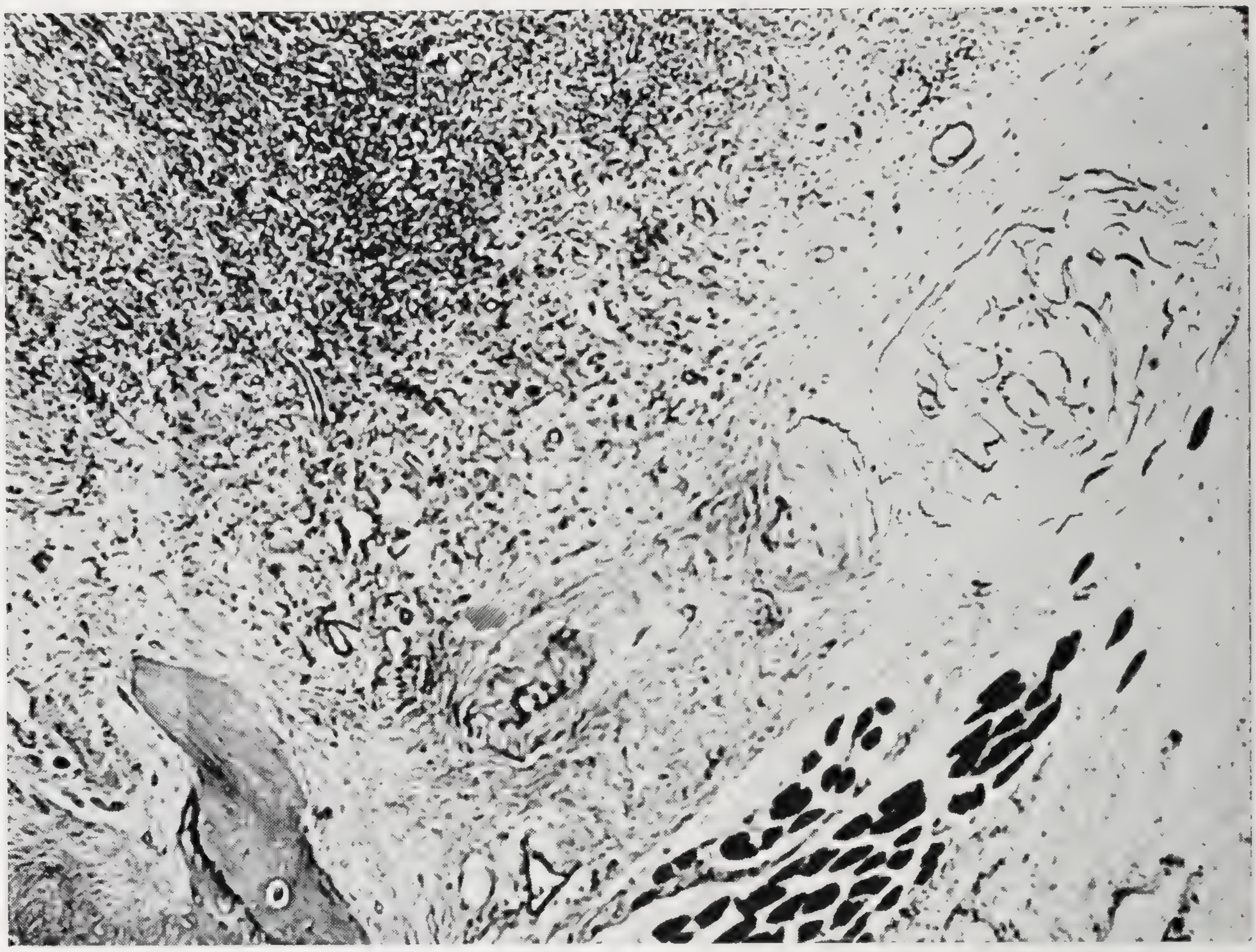
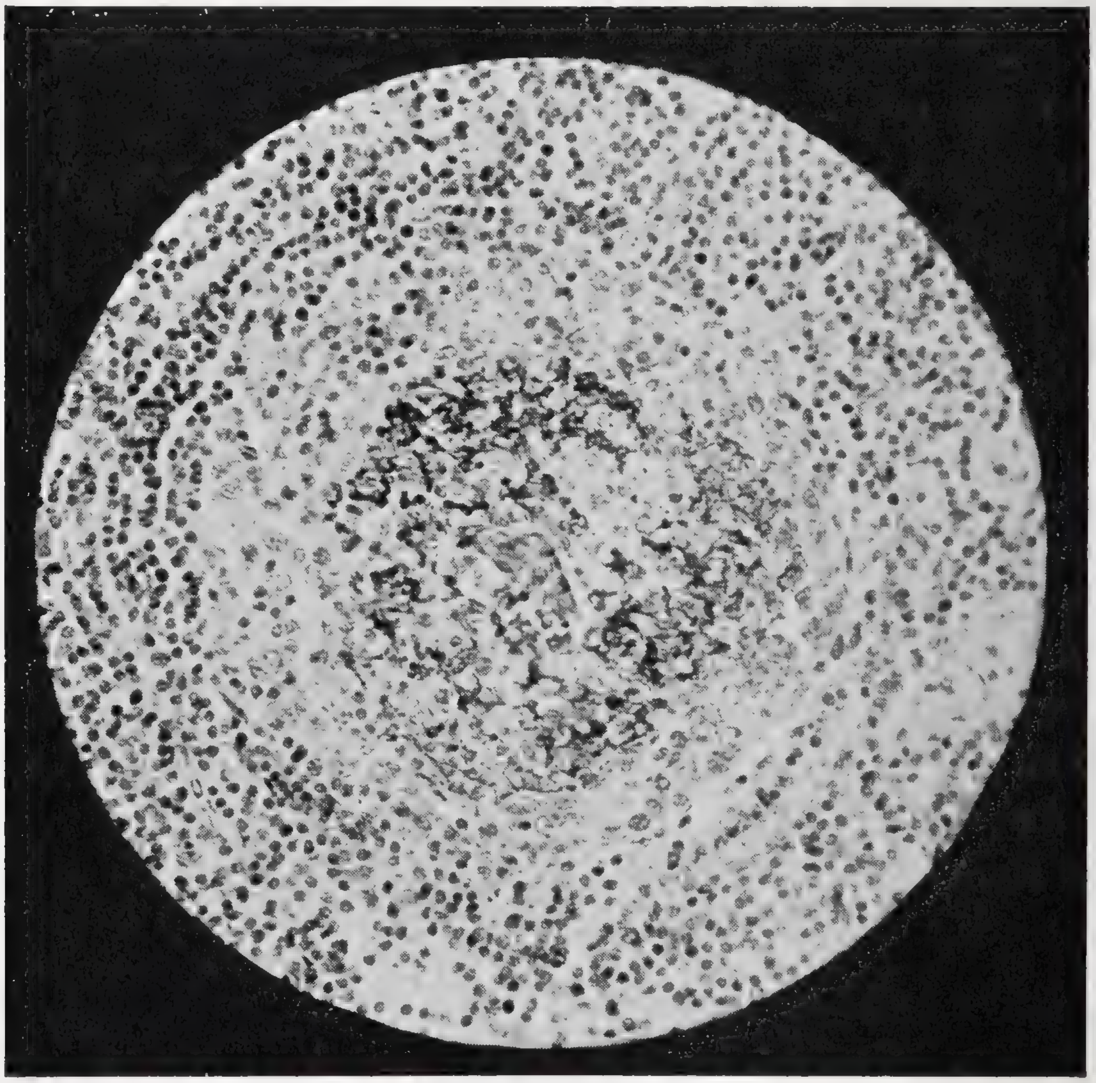


Fig. 1.



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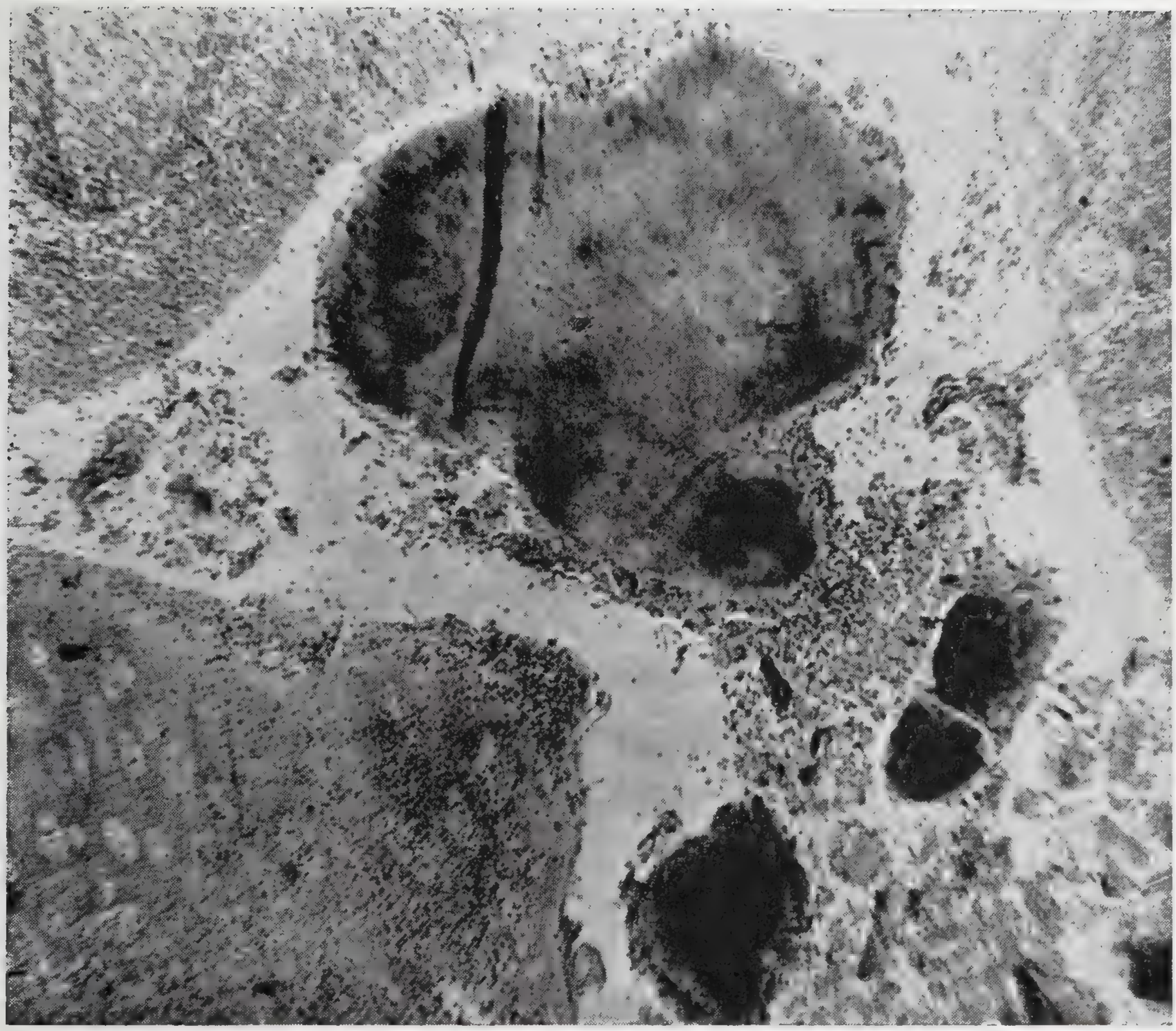

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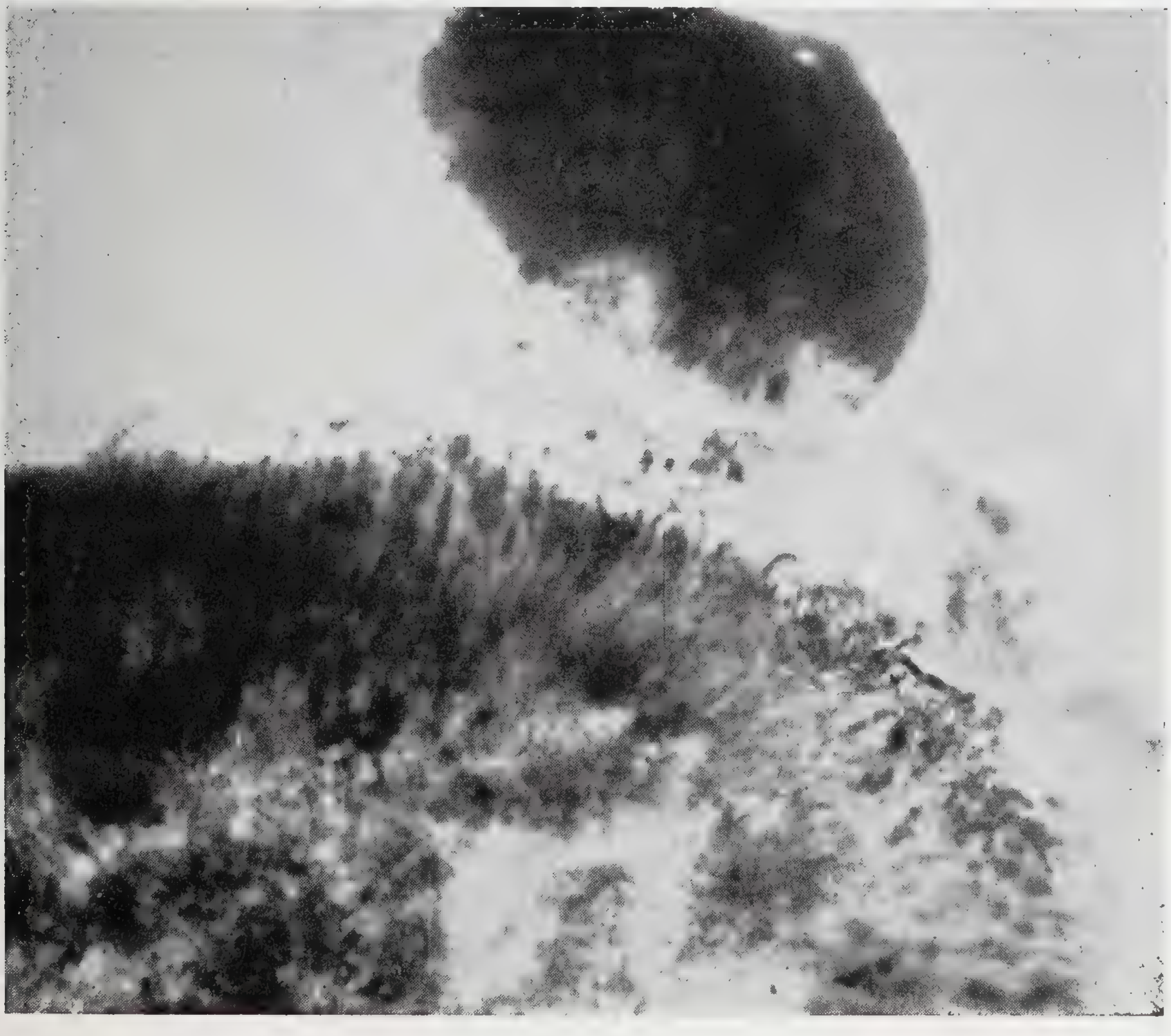

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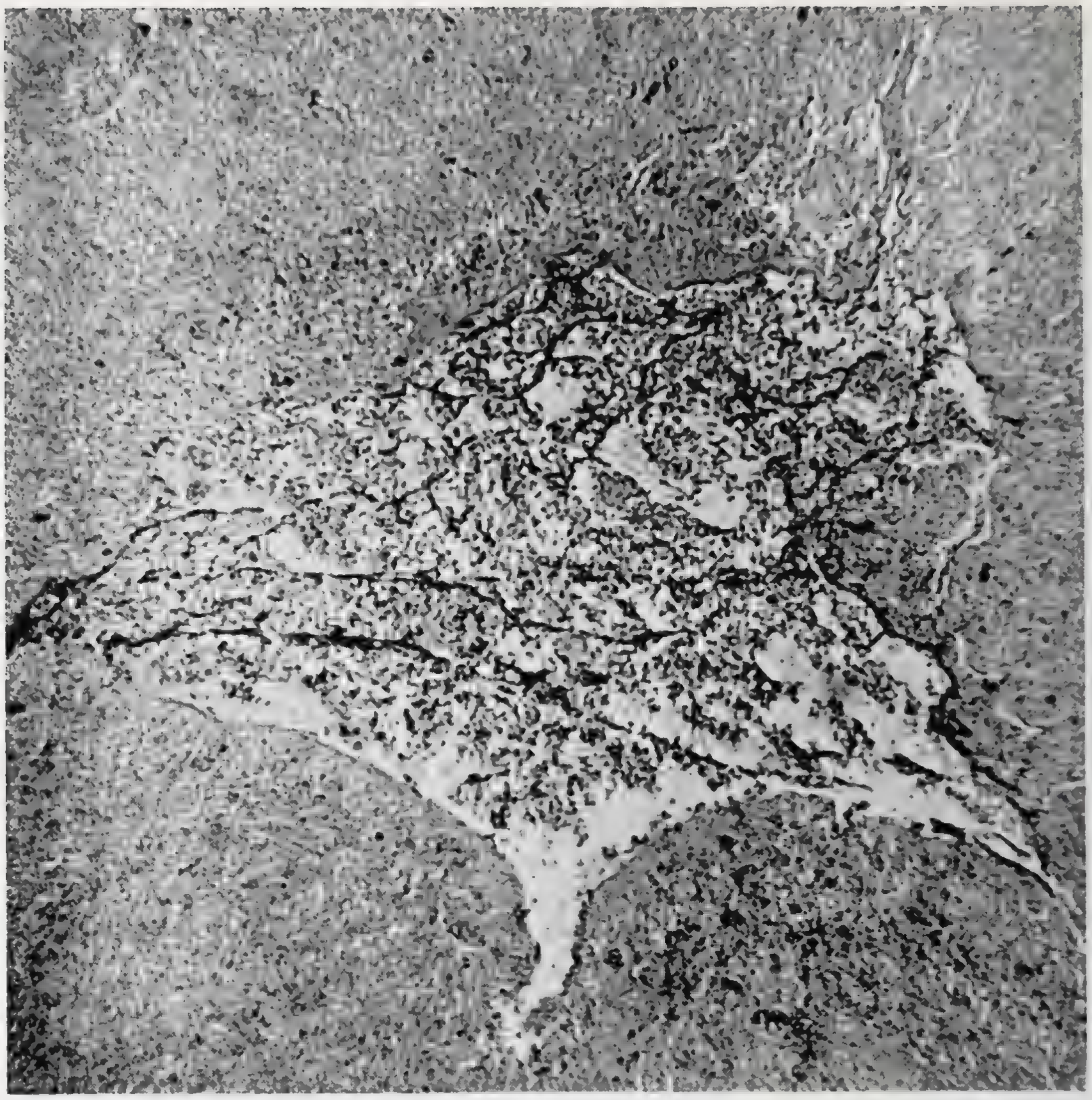

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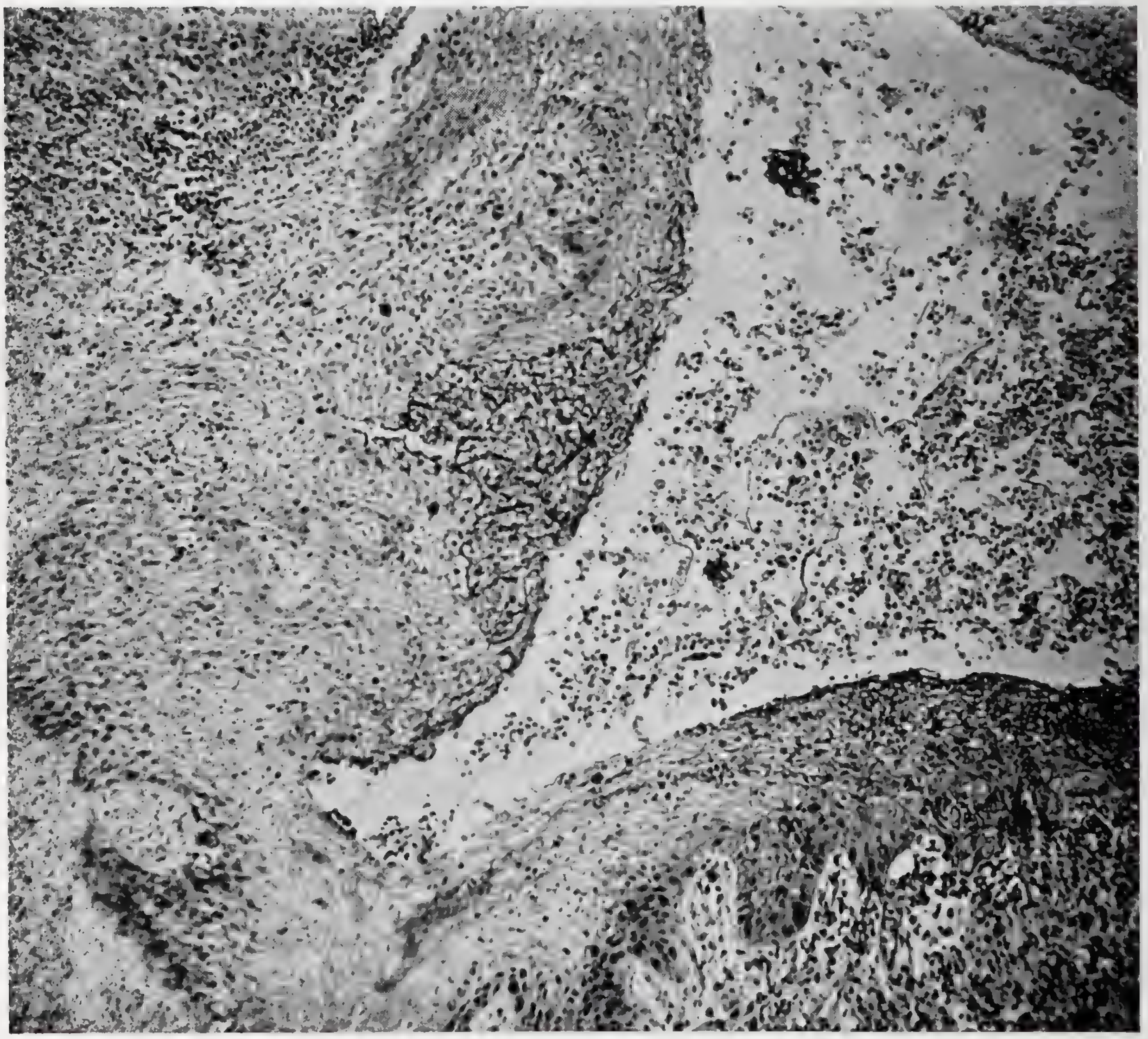

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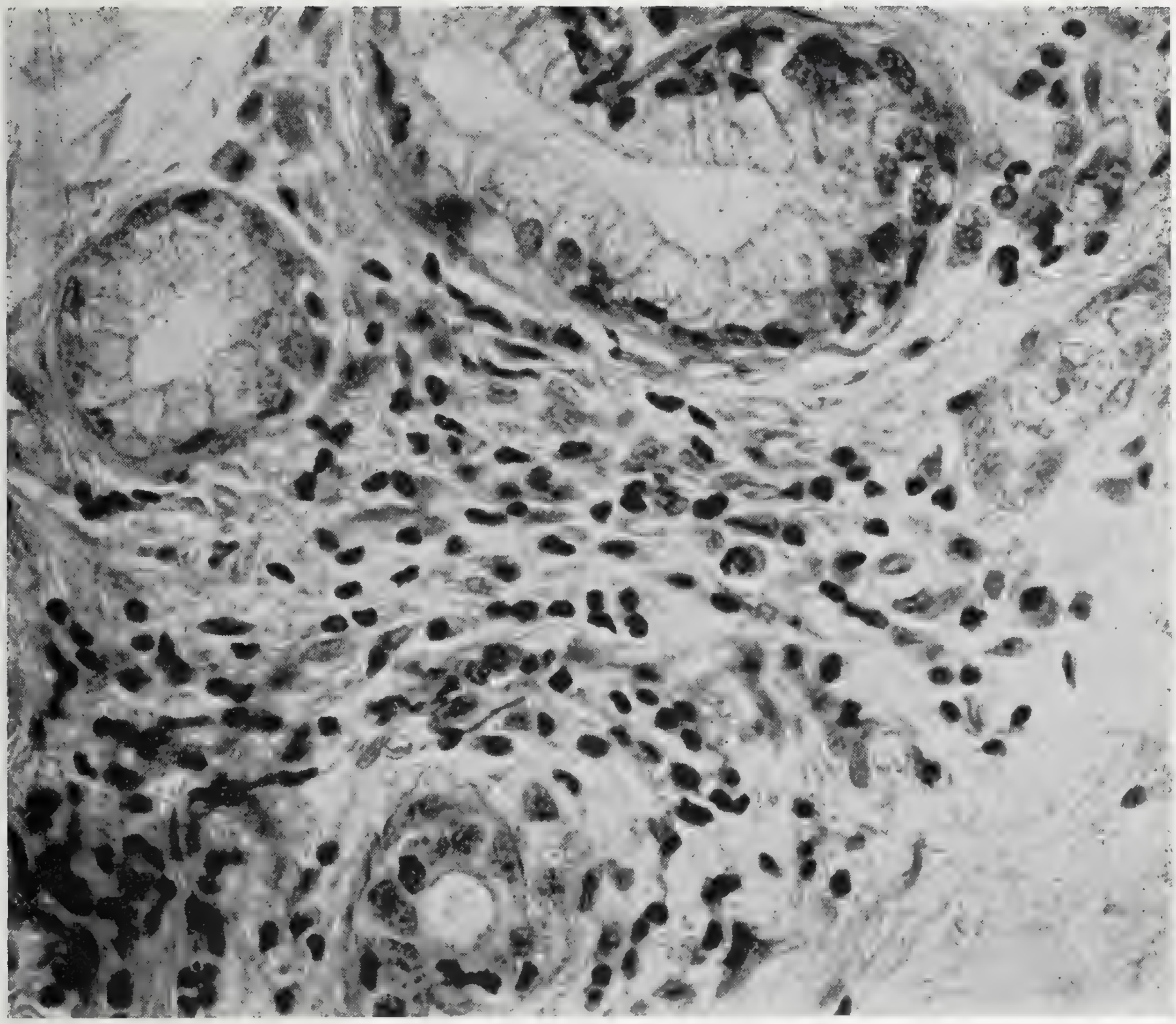

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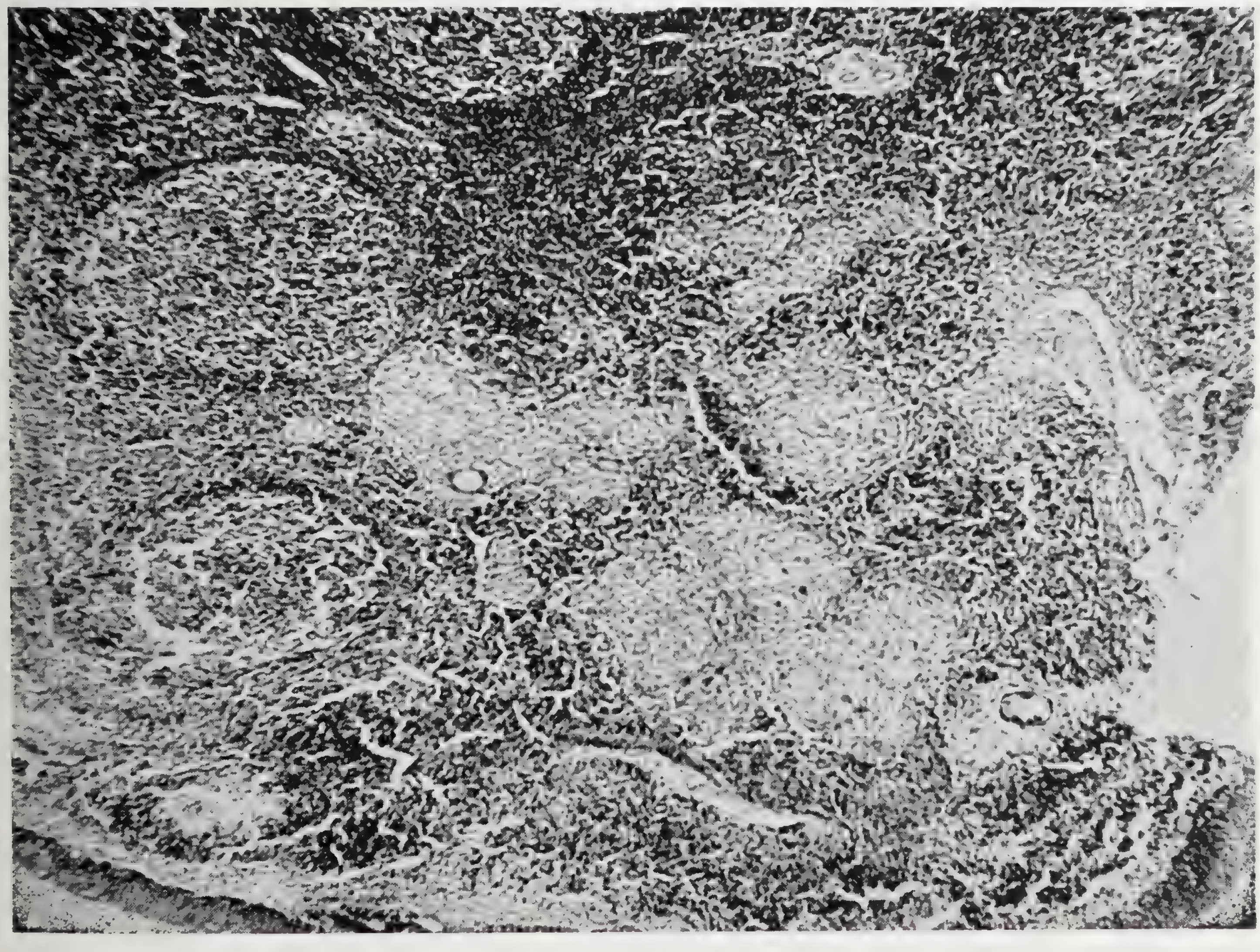

Fig. 8.

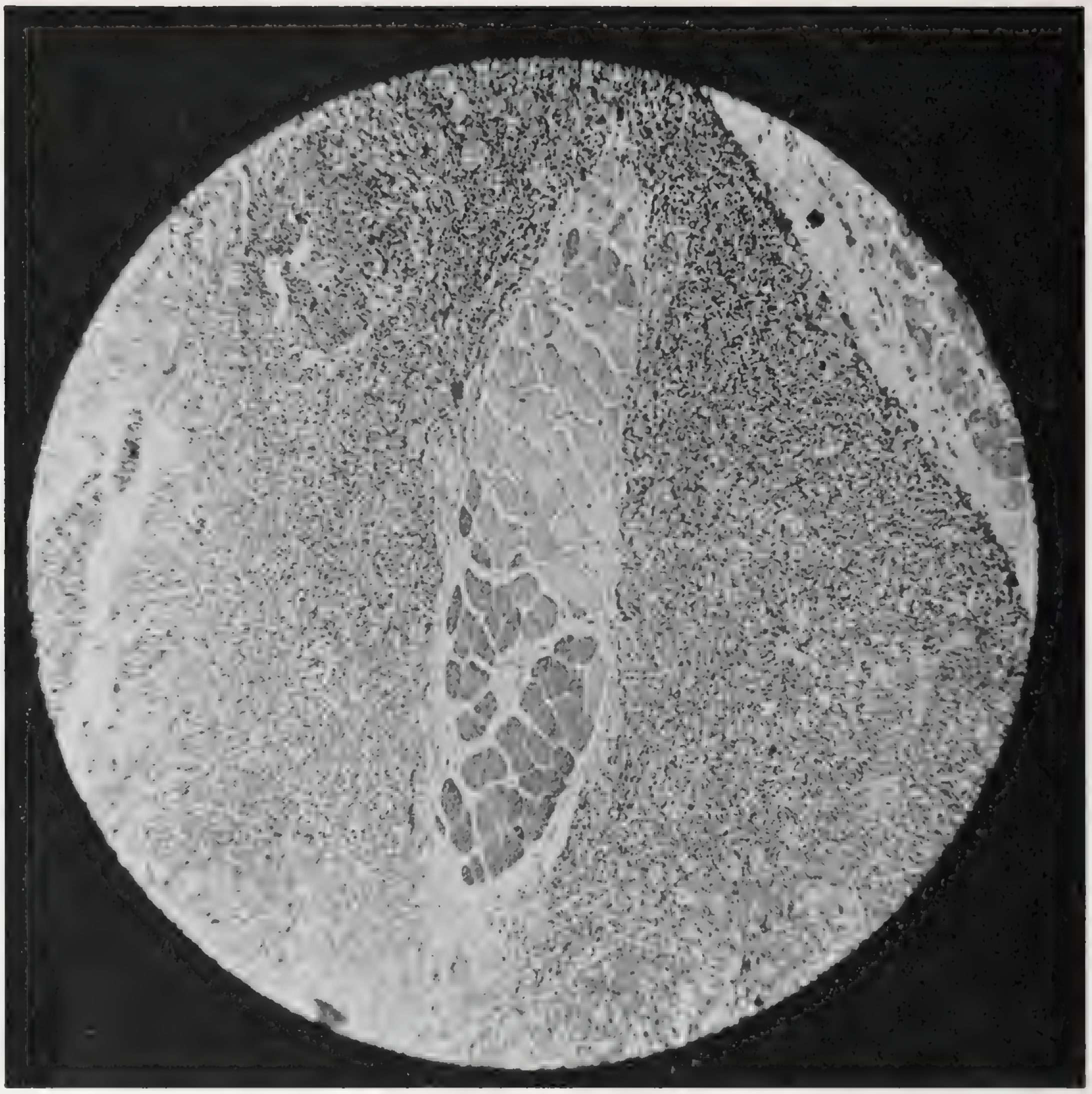

Fig. 9.

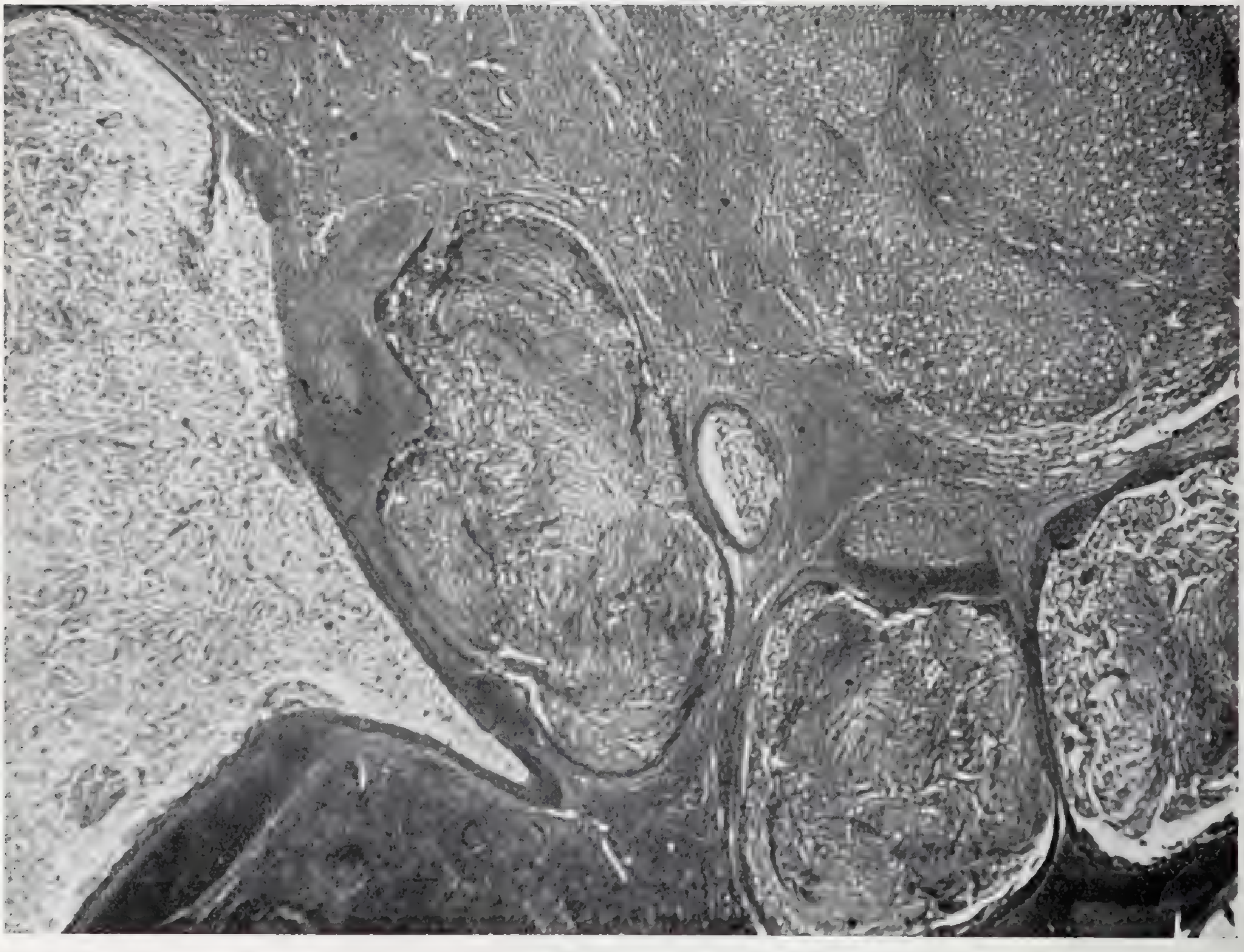

Fig. 10.

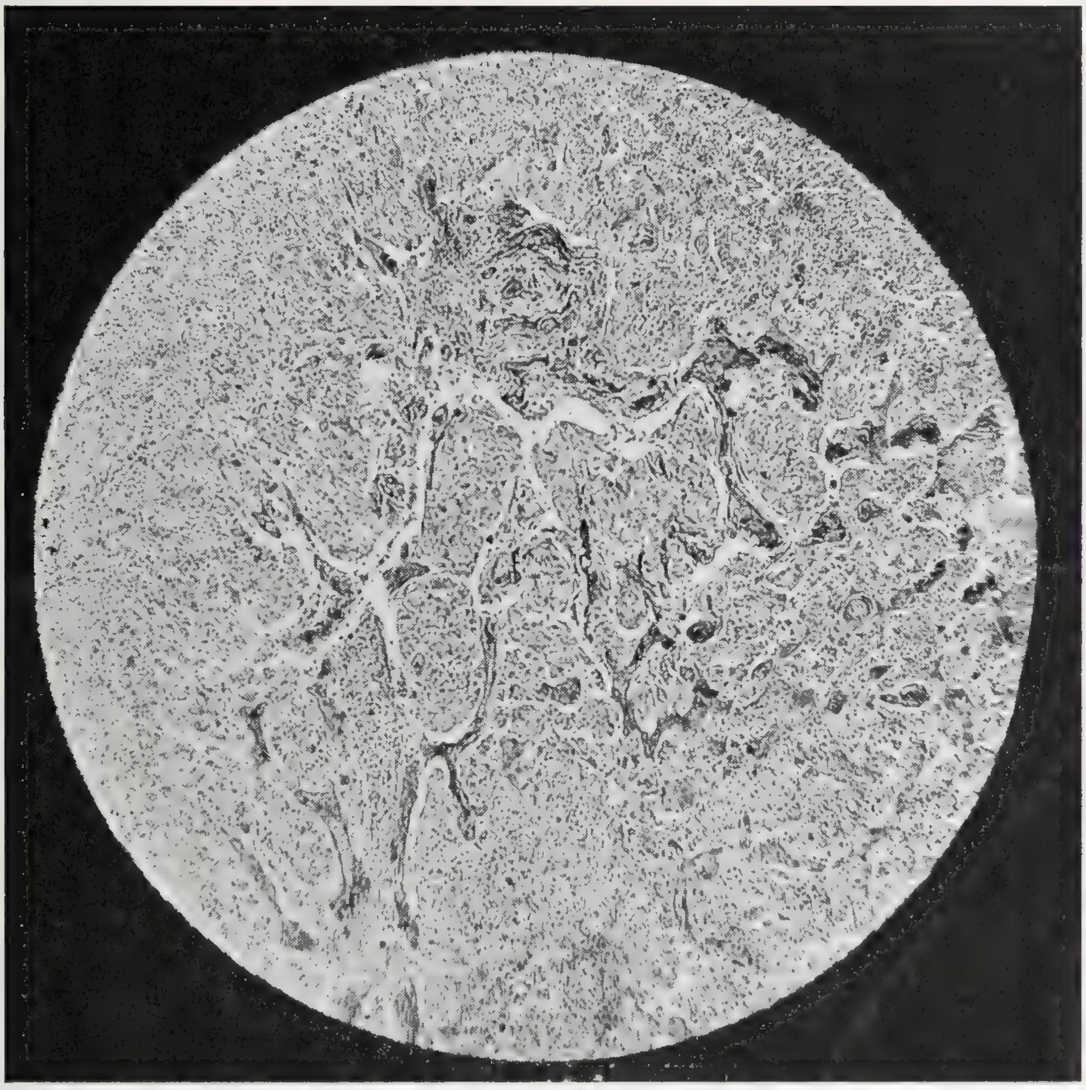

Fig. 11.

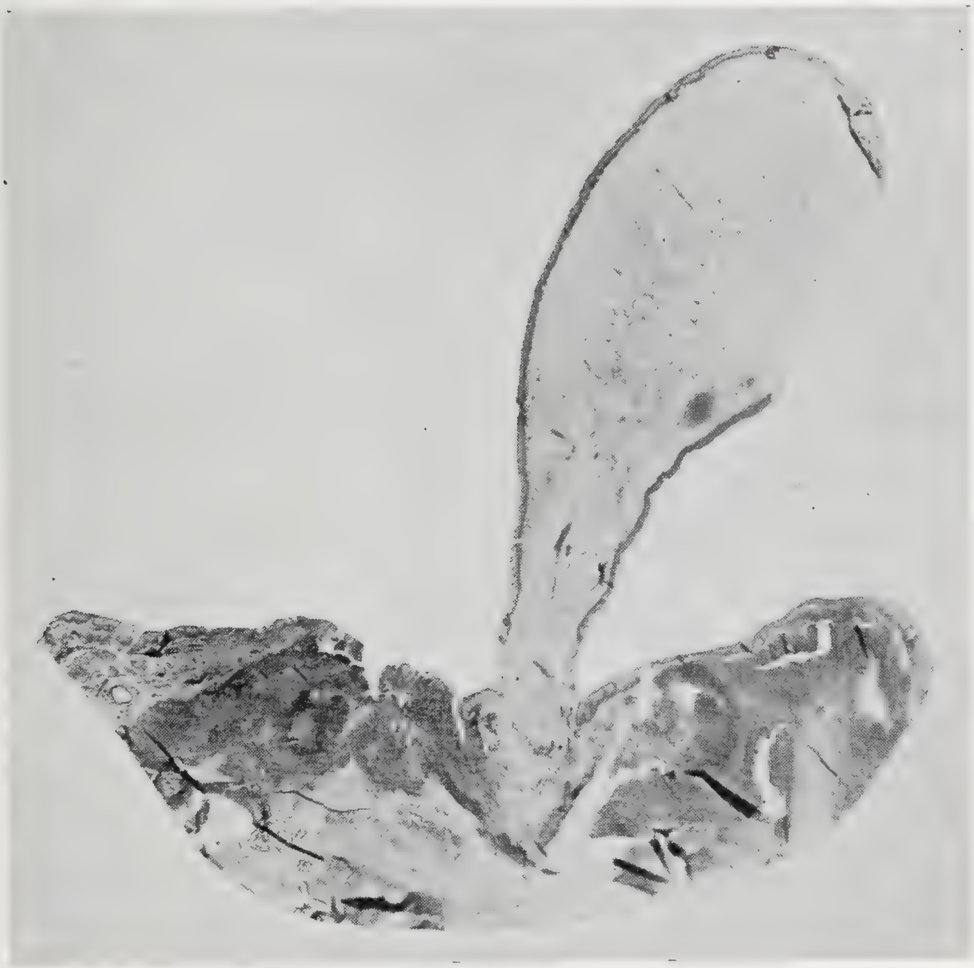

Fig. 12.

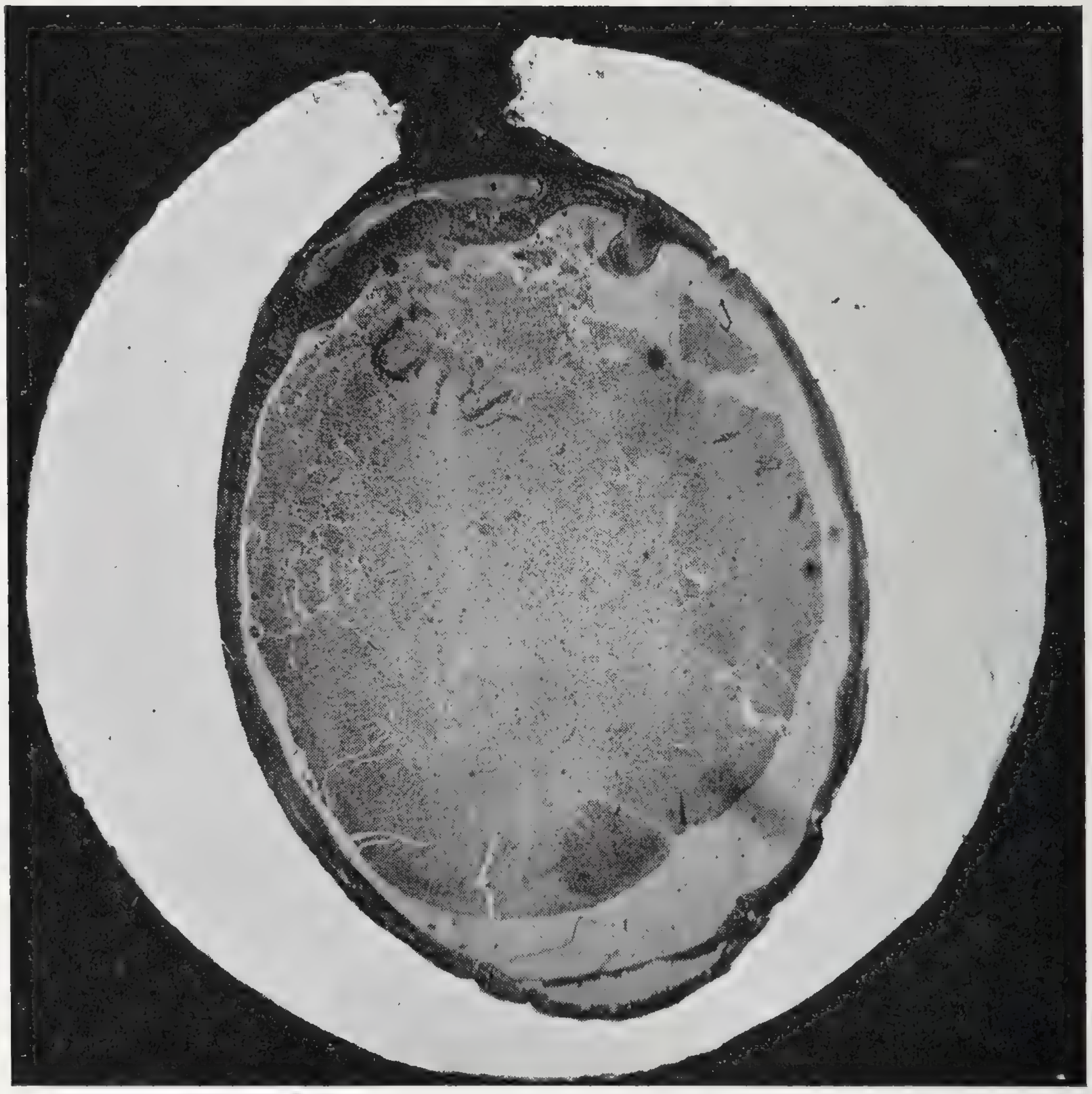

Fig. 13.

SOME OBSERVATIONS ON THE CULTIVATION OF TISSUES IN VITRO

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SOME OBSERVATIONS ON THE CULTIVATION OF TISSUES IN VITRO.*

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Through the work of Harrison of Yale, who demonstrated that tissues could be cultivated in media outside of the living body, a new impetus has been given to various cytological studies. In his early work Harrison demonstrated that portions of tissue isolated from embryonic nervous structures could be grown in the fluid obtained from the lymph sac of the frog. These implanted tissues showed, after several days, a development of prolongations from the isolated cells into the surrounding media. The cultures were prepared aseptically upon hanging-drop slides. Harrison found considerable difficulty in preventing bacterial infection of his cultures. He further found that although he attempted to deal with the nervous tissues from the medullary tube, in some cases portions of other tissues were also included in the preparation. This transplantation of several types of tissue led to some difficulty in indicating with any degree of accuracy the type of cell which showed proliferation in the prepared cultures.

The study of this material was confined, in the greater part, to observations made upon the nervous system. He found that the ordinary histological methods of dealing with adult tissues were inapplicable, owing to the extreme delicacy of the structures. For these reasons it was not possible to carry out the ordinary staining methods which are applied in the examination of adult tissues for the differentiation of particular types of cells or fibrils. Harrison confined his staining methods mainly to alcoholic hematoxylin. He found much difficulty in isolating and preserving nerve fibers which had developed in his artificial media.

The technic of Harrison was later modified by Burrows and

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the experiments were extended to other animals. Harrison found that the lymph from the frogs was not a very suitable medium to work with, as the coagulation of this fluid was not uniform. At times the lymph formed no clot or was much too soft to be easily handled. Burrows was able to substitute the blood plasma of frogs for the lymph. Later he was able to carry out similar experiments in using the plasma of chickens and inoculating this with the tissues from embryonic chicks. In using this method for the study of cell growth it was easier to carry out the aseptic technic than was possible with the frog preparations.

More recently this work has been still further extended by the experiments of Carrel and Burrows. These investigations have shown that by using a homologous plasma various tissues of embryonic and adult animals can be cultivated with fair ease. These authors claim to have grown connective tissue, cartilage, peritoneum, bone marrow, bone, skin, cornea, mucus membrane of the tongue, thyroid gland, spleen, suprarenal gland, kidney, pancreas, testicle and ovary from the dog and cat. At a later date they have reported the successful cultivation of human and fowl sarcoma upon the homologous plasma.

In the variety of organs from which authors obtained growth of cells, they claim they have observed the development of structures peculiar to these organs, such as tubules of the kidney. These authors were also able to obtain several generations of their cultures by transferring some of the material under cultivation to fresh media. They have also indicated that the cultivated tissue from fowl sarcoma will reproduce the tumor when inoculated into a healthy chicken.

Lambert and Hanes have used the method of cultivating tissue in vitro for studying cytolysins in connection with mouse and rat sarcoma. They obtained the interesting result that the mouse sarcoma grows vigorously in the plasma of normal rats, and that rat sarcoma is readily cultivated in the plasma of normal guinea-pigs. These studies indicate the important facts that tissues may live and grow in heterologous plasma. This, as we shall point out later, has been our own finding, in that we were able to cultivate guinea-pig and chick tissues in human plasma.

The development of these methods for cultivating tissues outside of the body suggests the solution for many problems in immunity and cytology. However, much has yet to be done in the development of a technic by which certain definite and recognized tissues may be segregated and individually planted upon media without the intermingling of cells of a different character. Besides this a new histological technic is required by which we may use, with some security, stains which will differentiate recognized tissues in the embryonic state which do not take the microchemical reaction as do the adult tissues. It is found, for instance, that the early embryonic connective tissue cells do not show fibers by the selective staining methods as do the fibroglia of the adult tissues.

In our own work, to be described, we have found the greatest difficulty, not in the cultivation of tissues from different organs, but in the determination of the nature of the cells which are reproduced. As is recognized by histologists, the differentiation by staining methods of various types of cells when these have been isolated from their surrounding structures, from other cells of an entirely different origin, is extremely difficult, and the difficulty increases greatly when we are dealing with the differentiation of embryonic or actively growing cells. Up to the present a definite differentiation of cultivated tissues has not been made, nor have the various features of evolution of the embryonic cells of any type been followed through to the mature stage of that cell.

Technic. — In our efforts to obtain actively growing cultures we have found certain things of great importance, and in order to obtain the best results certain rules of technic must be closely followed. Strict asepsis during the manipulation as mentioned by previous writers has been found essential. While not producing immediate death, yet the presence and growth of bacteria in a great measure retards and limits the life of the growing culture. Nevertheless the extent of harmful action depends largely upon the type of tissue and nature of the media used. In guinea-pig cultures we have found that various infections rapidly terminate the growth of the culture, while infection of chick tissue in chicken plasma simply retards and limits the growth. We have not studied the nature of these contaminating organisms.

In a general way our technic for preparing and inoculating media with living tissues differs little from that described by others, but a few points of importance in this respect may be worth mentioning. Tissues which are planted directly from a living animal or immediately after the death of the animal give the best results. It is essential to transfer these prepared cultures to the incubator immediately after they have been made and under these conditions evidence of growth is obtained in one and a half hours. It has also been found that tissues which have been teased from larger masses by means of sharp pointed instruments, just at the moment of inoculation, show a greater activity and respond more rapidly to growth.

For various purposes of experiment and observation we have selected different types of cultural methods. For observing, from time to time, the character and nature of the growing tissues on the warm stage, hanging-drop preparations have proved most convenient, while these preparations were also best adapted for purposes of staining and study of microchemical reactions.

In order to observe growing cultures macroscopically and for the purpose of transplantation, large plate cultures have been found best suited. The ordinary petri dishes, heavily coated about their margins with sterile vaseline or paraffin, are most convenient for this work. In this way considerable nourishment may be provided to the tissue, and under proper conditions a very luxuriant growth is obtained. The new growth may easily be distinguished by the naked eye in the halo effect or outer translucent margin as compared with the central opaque area of the original implanted mass.

In our selection of culture media we have found that different types of tissue grow best in different media. As mentioned by Burrows and later by Lewis, connective tissues grow best in the homologous plasma, and while we have observed epithelial-like cells in plasma media they have been found to grow more abundantly in an agar serum medium.

In using plasma media, only a sufficient amount for immediate use is prepared, and while it may be preserved for some time in a liquid state at a low degree of temperature it was found that the longer it was kept the more did it inhibit active growth. In order to simplify our technic and to secure better results, fresh serum was always used. The plasma was obtained from the blood of various animals by withdrawing a sufficient quantity of fluid from the veins by means of a sterile syringe. This process was carried out under all aseptic precautions and without the use of an anesthetic, which we believe would interfere with the quality of the serum.

The blood thus collected is received in paraffined tubes and chilled. The blood is then rapidly centrifugalized without taking any particular precautions for keeping the blood cool other than that the centrifuge be in a cool place. With an adequate centrifuge the clear plasma may be collected within two minutes. This clear fluid is then transferred by means of paraffin tubes into containers chilled in ice. Such plasma will remain fluid for hours and is easily handled for the purpose of making cultures.

It is always well to have on hand the materials and instruments necessary for carrying on the culture before the plasma is prepared. The plasma is then handled in paraffin coated pipettes and spread upon cover-slips or plates upon which the culture is to be made. The plasma which is placed upon the cover-slips will usually remain fluid for a few minutes, and should be immediately inoculated before the fibrin has formed. Thin cultures are best in that they afford better opportunities for staining and growing the cultures and are more easily examined under the microscope.

It is well to note in the preparation of plasma cultures that the coagulation time in different species varies somewhat, as for instance guinea-pig blood, even after thoroughly chilling, if left too long in the centrifuge, will develop a firm fibrin clot, while on the other hand chicken plasma does not show this tendency towards rapid clotting.

Serum collected in the ordinary way has also been used, but with only fair success. It was found that better results were obtained when the fibrin was present in the culture media. Probably the fibrin assists the growth in presenting a framework upon which the cells may develop, as well as affording useful nutriment when it is dissolved by the proteolytic ferment. In fluid serum fair growths of connective tissue were obtained, and these were always observed to be attached to the surface of the cover-slip.

Serum agar cultures were also prepared, one per cent plain agar was prepared with tap water without the addition of bouillon. This was put up in small tubes and when required was melted and again cooled to 40° C., when blood serum was added in varying dilutions. The best results were obtained when the serum and agar were mixed in the proportions of two to one or three to one. In this medium was observed a luxuriant growth of not only connective tissue fibrils but also epithelial-like cells. Bouillon was later added in various strengths to this serum agar mixture but with no distinct advantage. Carrel and Burrows were unable to obtain cultures on any serum media.

Staining. — The main difficulty which confronts the observer, after he has been successful in obtaining growths in his artificial media, is to apply a proper technic so that his cultures may be studied at leisure and that they may be kept for future reference and comparison. We have found that for the closer observation of cell structure it was best to stop the growth at the end of five days, before the degenerative changes altered the character of the cells. It was not uncommonly found that after six days there were various types of granules and globules within the cells, which, as we will indicate later, are an evidence of a lowering of the cell vitality.

Moreover, it is very necessary to obtain suitable methods by which a staining technic could be applied to differentiate cells of different orders. As has been reported by other observers it is impossible to observe mitosis of cells in the living cultures. We have on repeated occasions closely watched the indefinite nucleus, but were never able to observe any arrangement of the chromatin filaments. That mitosis occurs is only to be indicated in stained preparations.

It is also to be noted that the cells developing in cultures react somewhat differently from the adult tissues in the animal body. It was commonly observed that in cultures where the connective tissue had been most active in proliferating and in which the general appearance of the cells and the arrangement indicated a connective tissue character of the structure, we were unable to get the differential staining reactions for connective tissue fibrils. At times these fibrils were wanting and the cells possessed only coarse protoplasmic processes.

In general we have found that formalin fixation was the best. The small hanging-drop preparation was immersed in five per cent formalin and allowed to fix for about an hour. It was then washed in water and various stains were applied. We have also used the Zenker fixation, but this was not so uniformly successful. Precipitation of the chrome and mercurial salts upon the fibrin threads gave much difficulty in later removal and treatment. Moreover, the formalin prepared material permitted the demonstration of fat deposits, which were not as well indicated in the Zenker specimens. However, for special stains the latter method was always used. In the majority of cases we applied the stain directly to the culture. It is found that much better results were obtained where the culture was made on a thin medium so that the entire mass could be stained. This avoided the subsequent cutting on the microtome. Where serial sections were made from paraffin blocks we found that great alteration of the newly developed cells took place.

The delicate protoplasm of these growing cells undergoes much shrinkage when put through the usual imbedding methods for paraffin, and the staining qualities are also considerably altered. Sudan III. and hematoxylin have formed a most useful method. Besides this, Van Gieson's connective tissue stain was useful in demonstrating fibrils of the more mature connective tissue cells. Mallory's connective tissue stain was tried in the Zenker material but gave no result on account of the fibrinous medium. Eosin-methylene blue gave fair results.

As a considerable part of the newly developing cells grows between the coagulated hanging-drop of plasma and the cover-slip, there was no difficulty in studying the stained preparations with the highest powers of the microscope. Particularly was this true when the culture was made in a very thin layer of plasma. Such a stained and prepared culture may be permanently mounted by means of Farrant's solution without exposing the section to alcohol or xylol.

As mentioned by Carrel and Burrows successful attempts at transplantation of growing cultures have been made and some importance is laid on the fact that the new growth resulting from such a transplantation is somewhat different from that of the original mass of growing cells, in that the arrangement of the cells with their processes is not as compact as in the original growth. The new cell formations have become larger in size and contain many fat globules. We have found that the actual transference of growing tissue can best be accomplished by plate culture where the growth can be readily distinguished by the naked eye and the danger of planting cells from the original mass is avoided. Best results are obtained in transplanting from two-day-old cultures while the tissues are in an actively proliferating state.

We have found that cultures once showing evidence of growth can be safely examined from time to time for about four minutes in a room at a temperature of 28° to 32° C. without endangering the life of the growth.

RESULTS OBSERVED DURING THE GROWTH OF DIFFERENT TISSUES.

Connective tissue. — This type of tissue grew most abundantly and showed evidence of growth in the cultures of all the various tissues planted. On one occasion we have observed a growth twice the size of the original mass in three hours. Connective tissue shows an earlier attempt at growth than any other type of tissue and in the average drop of plasma growth usually lasts from fifteen to twenty days. This type of cell usually assumes a spindle shape and usually a radial arrangement from the implanted mass, and as the growth extends into the surrounding fields of media the fibrillar processes as well as cell bodies and nuclei become lengthened. The arrangement of these growing cells are in the direction of the fibrin threads in the plasma, to which they cling for support. Later, as the growth extends into the media, the cells form a network causing the protoplasm of the cells to become distorted into many shapes and forms, so as to assume, in some cells, the appearance of typical nerve cells. Many cells are connected to others by means of these fibrillar processes, some having as many as six to eight limbs. After four or five days' growth we have frequently observed a few cells to wander beyond the growing area and become separated from the general mass of growing cells. These segregated cells have been found to be more round or oval in shape, their processes gradually becoming shorter and the entire cell body much larger than those cells from which they have been derived. The nuclei of these cells also assume a round or oval shape, slightly granular and very sharply outlined, while in the protoplasm of the cells many fine and a few large granules appear.

In every growing culture of connective tissue we have observed, after four days, the appearance of fine granules arising about the nuclei. Later these become larger and fewer in number, some apparently coalescing with others and extending into the fibrillar processes. These granules are readily demonstrated to be fat globules by applying Sudan III. Such granules and globules are found only in tissues which are actually growing or at least carrying on an active metabolism in the medium. When once the culture begins to degenerate the outline of the cells becomes very indistinct, the protoplasm granular or containing vacuoles, and the nuclei lose their prominence and become fragmented.

Many cells, especially those which are embryonic in type, not infrequently have two or more nuclei, but few nuclear figures were observed.

It has been our repeated observation that mitotic figures are not as commonly present as might be expected. In examining cultures at various periods, from those only a few hours old to those which had grown for some days, we were much astonished that although there was an unquestionable increase in the number of cells in the media, only rarely were nuclear figures present. We have repeatedly observed that the nuclei of the cells showed stages simulating closely a process of amitosis, some nuclei showed bilateral indentations like the figure of eight, others were lying side by side with only a short link of nuclear material between them, while again many cells were observed with two distinct nuclei lying close to each other. It should be pointed out that in the older cultures and more particularly in the mass of the implanted cells, various nuclear changes indicating degeneration and fragmentation are found; these, however, differ much from the character of the nuclei seen in the growing cells.

The protoplasm of the young cells is clear and almost homogeneous; there are no granules save the appearance of a very fine stippling. No structure can be made out in the protoplasmic body.

As has been pointed out by Carrel and Burrows, the growing cells of connective tissues commonly have a radial arrangement. This arrangement is mainly determined by the direction of the fibrin threads along which they grow. In an active growing culture of connective tissue there is commonly found a development of a clear area like a halo surrounding the original mass. This halo develops with the increase of the growing cells, and probably is the result of a lytic substance acting upon the formed fibrin. It is probable that a part of the ferment acting upon the fibrin is derived from the dead cells or the cells undergoing destruction in the original implanted mass. Commonly the liquefaction of the medium takes place at a later period than the production of much of the new growth. We must say, however, that in no case have we observed the development of the circumscribed destruction of fibrin in preparations showing no growth of the implanted tissue. When the area of liquefaction is well advanced, the connective tissue cells continue to grow towards the periphery, but not with the same definite arrangement as the early cells growing along the fibrin threads. When the cells reach the border of the liquefied area they tend to grow along it so as to form a new zone or "shore line," encircling the liquid medium.

The morphology of the growing connective tissue cells differs very widely from those of the adult animal. Nevertheless the character of the growing cells is fairly constant even when obtained from different animals (chick and guinea-pig). On morphological grounds alone it was often difficult to be convinced that the cells were of connective tissue origin. The early development of the cells from the implanted tissue showed mainly spindle types, having a fairly large body and ending at both extremities in fine filaments which were attached to the succeeding cell. At times, also, filaments extended from both poles to cells which lay laterally. These filaments formed definite attachments to those cells which presented the progeny or the parent. In this way the chain or chains of cells arising from a single unit could be followed. In this way, too, many cells though morphologically different from other cells in the chain could be recognized as belonging to a certain type.

Similar to the observations made by Harrison upon nerve cells, we are led to believe that the large processes and fibrils of connective tissue are formed by the drawing apart of two cells which were first joined by heavy protoplasmic processes. Thus in the growth of new cells the progeny wander away from the parent, but still remain attached by linking fibers.

Not infrequently the cells lying more peripherally formed large syncytial masses with large filaments and several nuclei. Again others were observed with a more spherical body having one or two large protoplasmic offshoots and smaller thread-like filaments. Morphologically these resembled nerve cells but did not contain granules in their protoplasm.

In the multiplication of some cells long ribbon-like processes are produced which sometimes end in oval masses or islands of cells which are closely apposed to each other. The individual elements of these islands are more or less circular without evidence of fibril production.

In the older cells (over ten days' growth) it was possible to obtain the reaction for connective tissue fibrils by the Van Giesen stain. By this method it was found that the cell processes stained uniformly, and did not show the presence of multiple fibers. There was no evidence that the cells produced more than one fiber in each prolongation, but at the same time a single cell could produce many processes from its periphery. By the traction of several attached cells, in the same direction, a number of the fibers may become arranged in closely aggregated bundles looking like a single process. We were unable to obtain results with Mallory's stain.

As was observed above, the young growing cells are devoid of granules in their protoplasm. As, however, these cells become older, granules appear occupying the cell immediately about the nucleus. Later, similar granules are found extending into the processes and much of the cytoplasm is filled with bright refractile bodies. Similar bodies have been described by Carrel and Burrows. In the cultivation of tissues these granules indicate degenerative changes in the cell and are found to consist of fat. Beautiful preparations may be obtained by staining these tissues with Sudan and hematoxylin. That these granules indicate a process of degeneration and are dependent upon an altered condition of the foodstuffs is shown by their early appearance in the cells close to the original mass which was inoculated. The peripheral cells are usually last to show this fatty degeneration.

An interesting question arises as to the origin of this fat. From our observations we would conclude that it is derived from the presence of invisible fatty compounds contained in the plasma. The quantity of fat which becomes visible in these cells is, however, very remarkable. A small quantity may also be derived from the destruction of some of the originally implanted cells. We have found, however, that the total fat is not obtained from this source, as in those cultures which did not grow no considerable quantity of fat became apparent, either in the medium or in the dead cells which had been introduced.

The fat in the connective tissue cells first appears in fine globules which later coalesce to form single or multiple large globules. We have never observed the accumulation of fat in these cells to simulate all characters of a true fat cell. Moreover, fat in any quantity was never observed to accumulate in dead cells, though these may sometimes show the presence of very minute Sudan staining granules.

In a few instances we have observed the presence of cells of a signet-ring character closely resembling true fat cells. These, however, were not connected with the cells of the fibrous tissue type, and of their origin we cannot definitely speak.

Transplantations are quite readily made from actively growing cultures to fresh media, and by careful preparation successive transplantations could be carried on for many generations. In the new cultures just prepared active growth took place within an hour after inoculation. The morphology of the cells and the growth is found to be similar to that of the original culture, except that the individual cell bodies appeared larger and the arrangement of the colonies was looser. Fat also appeared in these cultures after the fifth day.

Connective tissues grow fairly well in alien plasma. Upon inoculating human plasma with embryonic chick tissues, we obtained a rapid formation of fibrin radiating in very thick and heavy layers from the original mass of tissue. Actual growth did not appear until seventy-two to eighty-four hours after inoculation, after which time the connective tissue cells grew to the extent of twice the radius of original mass in forty-eight hours, and then gradually ceased growing, becoming granular. Upon staining this growth the cells were found very indistinct, owing to the granular condition of the media which produced a cloudy appearance of the field. This cloudy condition of the media was still more marked in human plasma inoculated with guinea-pig tissue.

Liver tissue. — Cultures of liver tissue were obtained with fair success. Occasionally connective tissue fibers were the first to grow and appeared in the early hours of the culture. The growth of the cells from the parenchyma began later, usually not before forty-eight to seventy-two hours after incubation. When the growth of the liver cells once began they continued to increase and became widely scattered in the media at some distance from the original mass.

The liquefaction of the media about the growing liver tissue was commonly observed, and it was in this fluid medium that the greater number of new liver cells were to be found. In all the cultures of liver it appeared that the growth of connective tissue fibers and that of liver cells was quite independent. In no case did the liver cells arrange themselves in any particular system along the fibrous tissue.

These liver cells were of a fairly constant shape and of a uniform size. They appeared spherical and were much larger than the cells of the parenchyma which were introduced into the medium. The nucleus was usually centrally placed, but as the cells grew older the nuclei became eccentric and in the later stages of degeneration were even peripherally placed. The nuclei were fairly large and distinct. The protoplasm was clear and without granules until in the stages of degeneration globules of fat of various size began to appear. In cultures ten to twelve days old great numbers of small and large globules appeared in the protoplasm and gave the usual reaction for fat with Sudan. In observing the development of these fat granules it appeared as if some of them coalesced to form the larger globules.

In no case did these cells show the development of fibrils or protoplasmic processes. Other investigators have claimed to have observed the development of columns of liver cells growing from the original mass of liver tissue. This we have failed to observe in our cultures, save that in some instances small projections of cells were seen jutting out from the tissue implanted, but in all probability representing some of the original tissue. These projections could not be distinguished as growing cells and the accumulation of fat granules was different from that in the observed growing cells. That one does occasionally observe liver cells lying side by side is obvious, but such an arrangement is quite different from that of a liver column.

Similar difficulties present themselves in the recognition of growing liver cells as differentiated from other types of tissue. Although the cells were morphologically in relative size, shape, and arrangement, and in the character of the fat globules present, similar to liver cells in the adult body, the actual microchemical proof of their nature was not forthcoming. We had hoped that by the introduction of hemoglobin into the culture medium we could obtain some reaction in the medium indicative of liver cells, but our results proved negative. There was no evidence that the liver cells growing as isolated units in a hemoglobin medium could make use of this material.

Kidney tissue. — Our results in the cultivation of kidney tissue have not been uniform. The character of the growth resembled to some extent the results obtained in the cultures of liver tissue. In the cases where growth was observed the development of the radially arranged connective tissue fibers preceded the evidence of proliferation from the parenchyma. The parenchymatous cells developed as individual or isolated clusters of round cells in the clear medium beyond the implanted tissue. The individual cells were smaller than those of the liver and the protoplasm was finely granular. There was no great tendency on the part of these cells to accumulate fat, and the granules which did develop remained quite small. The individual cells showed little tendency to remain associated with others of the same kind, and we never observed any relationship in the arrangement of the parenchymatous cells with those of connective tissue origin.

Occasionally tubules were observed along the margin of the implanted kidney substance. These tubules we believe were structures which had become loosened from the edematous implanted tissues, giving an appearance of growing tubules. In no instance has a tubular formation been observed in cells quite free from the original tissues.

Spleen tissues. — Very fair results were obtained in cultures of spleen both upon a plasma and a serum medium. These cultures showed the development of great numbers of round cells varying in size. Not infrequently these cells formed masses or clumps of cells in the clear medium and in this way there were developed new foci from which active growth took place. The majority of the cells were of the size and appearance of large and small lymphocytes. The nuclei were round, sharply outlined, and stained intensely. There was no evidence of the production of fibrils, and the cells even in the older cultures showed no tendency for the accumulation of fat. In the majority of cultures from this organ there was a development of connective tissue. This tissue had the characters as previously described and showed the accumulation of fat in its protoplasm.

Muscle tissue. — In many cultures of heart muscle, cells were found to migrate into the surrounding medium from the original mass but without evidence of growth, while the supporting connective tissue grew quite abundantly. In a few instances the development of large syncytial branched cells having multiple oval and parallel arranged nuclei was observed. Such cells had the appearance of teased out heart muscle, but the protoplasm was of a more translucent character. In the early cultures there was no evidence of granules or globules in the cells. Later a few small globules appeared in the protoplasm. The further growth of these cells was one of increasing the size of the syncytial mass or at times the development of new protoplasmic processes. No fibrils simulating those of connective tissue were observed in these syncytial structures and the tissues stained yellow with Van Giesen.

Burrows has previously obtained successful cultures from heart muscle and claims to have noted the rhythmical contraction of these newly developed cells. This has not occurred in our cultures, although the implanted portions continued to beat for some time.

In the heart muscle cells which we believe were growing there is no evidence of transverse striations. It is therefore with some doubt that we mention these cells as definite muscle tissue. With, however, the difference in the morphological characters and in the staining qualities from the other connective tissues which we had observed, we were fairly convinced of the muscle origin of these cells.

It is to be noted that not uncommonly a number of striped muscle elements migrate from the original tissues at some distance from the culture and undergo degeneration without proliferation in the medium. These cells are not to be mistaken for the growing elements of successful cultures.

Epithelial tissues. — As previously stated the surface epithelial tissues were found to grow more rapidly and abundantly in a serum agar medium. Where the growth of the epithelial tissues predominates over the growth of connective tissue arising from adherent masses of corium, the cells take a radiating arrangement from the original mass and grow in several planes. The majority of these cells present an oval appearance, some containing one, others two nuclei and have a fairly clear protoplasm. Other cells are distorted into many shapes and forms and are of various sizes, as if in a process of division. The cells lying in the periphery of the culture are usually individual or in pairs, and contain fine fat globules, while those about the margin of the implanted mass show a great accumulation of fat in the protoplasm of the cell.

In these cultures of skin tissue we have not observed any chemotatic attraction between the connective tissues and epithelial cells. Each type of tissue appears to grow independently of the other.

In only one instance did we find that the epithelial cells took an arrangement to the growing connective tissue. In this case the connective tissues which grew abundantly formed a well developed membrane about the liquefied area of the plasma, while epithelial cells were compactly arranged upon the inner side of the stroma. There was, however, no evidence of a Malpighian layer. Care must be taken to exclude the migrating epithelial cells from the consideration of true growth. Intercellular bridges or the presence of keratinizing cells were not observed in any cultures.

Degenerative changes were evident in some of the epithelial cells in the presence of very minute fatty granules.

During active growth mitotic figures were quite evident in some of the epithelial cells.

DISCUSSION.

The cultivation of tissues outside of the body offers an excellent means for the study of the cytology of tissues, as well as of the metabolic activity of the cells. With the technic that is now established the difficulty of obtaining growth of diverse tissues upon different artificial media is no longer very great. The results of the different investigators indicate that almost every kind of tissue of warm-blooded animals can be cultivated in vitro. Nevertheless considerable work is still necessary for the development of a microchemical or other test to conclusively prove the nature of the different cell elements which are present in each culture.

In our own experiments we have found that our greatest difficulty lay in the actual determination of the nature of the cells which had grown. Similar to the experience of Lewis and of Lambert and Hanes we found that various tissues grew quite readily in serum media, heterologous plasma, and in simple solutions, as Ringer's preparation. It is evident, however, that the best growths are to be observed in the plasma media as described by Carrel and Burrows. It is of advantage to obtain this plasma from animals without the use of an anesthetic.

When the plasma media is inoculated with a tissue for cultivation, there is a rapid formation of fibrin which radiates from the point of implantation. This fibrin is of definite value to the growing cultures, in that the cells find support and are guided in their direction of growth. This has been very evident in all the cultures and particularly where connective tissues have actively grown. One is reminded of a similar process which is to be observed in the healing of wounds.

Another interesting point which simulates conditions arising in vivo is the migration of cells during the time that they are actively proliferating. It is not an unusual observation to find the new cells wandering at some distance from the parent cells and setting up new centers of growth. This characteristic of wandering is to be observed in almost all tissues and quite markedly in the epithelium of the skin tissues. It is probable that a similar migration of epithelial cells occurs on the surface of large wounds and that the new islands which form are from such points of migration. In cultures one must differentiate active migration of living cells from the passive migration of various materials and cells as is observed in the penetration of agar by red blood corpuscles lying on the surface. Similar observations were made by Carrel and Burrows.

As far as we have been able to observe, the connective and spleen tissues of adult mammalia grow most easily. On the other hand, no difficulty was experienced in growing liver and epithelial tissues from embryonic chicks. In the latter, however, it was also true that the connective tissues grow most profusely in cultures of all organs.

The actual evidence of growth is best observed in the comparison of the number of cells implanted with the number of cells present after several days' incubation. Little difficulty is experienced in recognizing a culture which has not grown, for in it the tissue shows little or no change save the disintegration of the nuclei and of the cells. In the cultures which have grown the number of cells increases many fold, and these new cells which constantly extend the circumference of the tissue mass show all evidence of vitality and of carrying on individual cell metabolism. These cells, however, differ from those which constitute the cells of the body in that they show no relationship in their metabolism or functional activity with other cells. Besides this we have never observed the dependence of growth of one type of cell upon another order of cells. Thus the development of cells of a glandular or epithelial origin is not associated with a preceding or simultaneous growth of stroma, so that epithelial tissues do not simulate the arrangement and structures of the tissue from which they were derived. It is obvious that the growth of these cells is very different from that in the body and that these cells must adapt themselves to circumstances quite different to that which we appreciate as the normal. In the method of preparing artificial cultures both the quantity and quality of food supply offered to the cells is arbitrarily the same. It is impossible thus to obtain for the different cells the preformed nutritive materials which characterize their metabolism in the body.

It would appear from this that the growing cells in vitro become isolated entities like so many amebæ differing only from each other in the degree of specialization, which they have acquired prior to their growth under artificial conditions. It is this increased specialization, which continues in the developing embryo, which increasingly offers greater difficulty in obtaining cultures in vitro. The results of successful cultures of any tissue may be compared with the autochthonous growth of tumor. Whether the comparison can be carried still further is yet to be determined. In a single instance we have obtained negative results from the implantation of actively growing embryonic tissue cultures into the homologous animal. It does, however, appear possible that the gradual adaptation of these cells with vegetative qualities by repeated transplantation on artificial media may so impress them with these qualities that independent and unlimited growth will continue when they are transplanted into an animal.

In all of our observations attention was paid to the presence or absence of function in the growing cells. Up to the present we have not been able to observe that any of the tissues carry out any of the functions which are normally allotted to them in the mature animals. Thus, although the liver and connective tissue cells show an abundance of fat globules, we could hardly indicate that this metabolism was carried out for a definite purpose by the cell. Moreover, the liver cells when grown in presence of hemoglobin did not appear to be able to make use of this. Likewise in the culture of adult tissues where the new cells were derived from specialized organs having previously acquired a specific function, the new progeny appear to exert their entire energy in vegetative activity without continuing their specialized function. It is probable that before we are able to have specific tissues functionate during artificial cultivation, we must properly modify the culture medium to serve the necessities of the cell. Others have already shown that the modification of the media by methods of dilution has a decided effect upon the rapidity of the growth.

One of the most interesting features of these observations has been the study of fat deposits in the various cells. Liver and connective tissues always accumulate fat in the greatest quantities. Fat usually appeared about the fourth day of growth and continued to accumulate during the life of the cell. The origin of this fat is not clear, for the amount that is seen in cultures of eighteen days' growth appears much larger than that which would be expected in the plasma or cells of the implanted mass. From the results that we have obtained up to the present the fat is of a semifluid nature and consists of a neutral compound.

Studies are at present in progress to determine the exact nature and origin of this substance.

[My sincerest thanks are due to Dr. Oskar Klotz for his supervision and assistance in this work, and to Dr. S. R. Haythorn for the photographs.]

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DESCRIPTION OF PLATES.

Plate VII.

Fig. 1. — Growth of connective tissues forming layers and connecting strands.

Fig. 2. — Growth of connective tissues forming fibrils and strands.

Fig. 3. — Growth of isolated connective tissue cells showing presence of fibrils and fat globules.

Plate VIII.

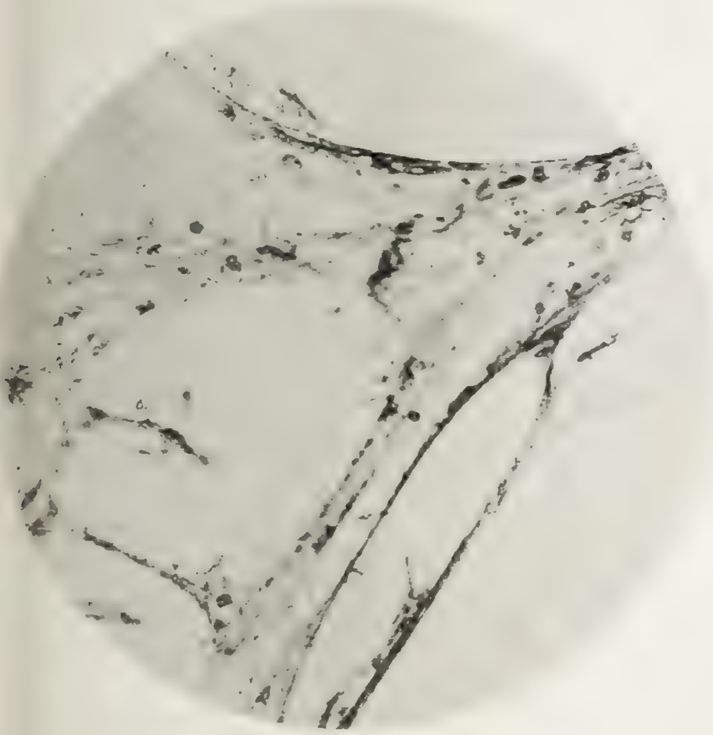
Fig. 4. — Newly developed connective tissue cells beginning to accumulate fat (six-day old culture).

Fig. 5. — Growth of skin tissues. The tissues shown are all newly developed and at some distance from the implanted mass. The single strand shown consists of connective tissue. The denser mass is composed of epithelial cells.

Plate IX.

Fig. 6. — Skin tissues from specimen shown in Fig. 5. The relation of the growing epithelial cells to the strand of connective tissues is shown.

Fig. 7. — Liver cells loaded with fat (fourteen-day old culture).



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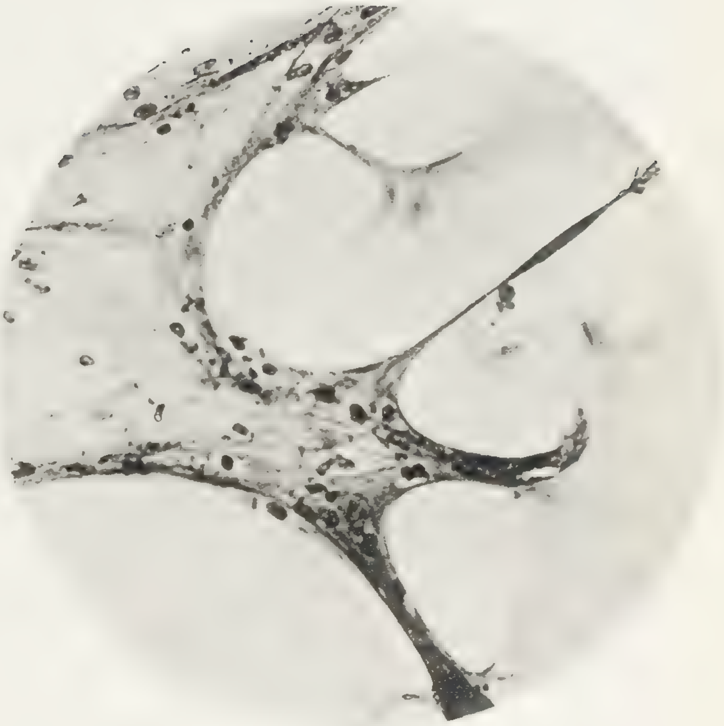

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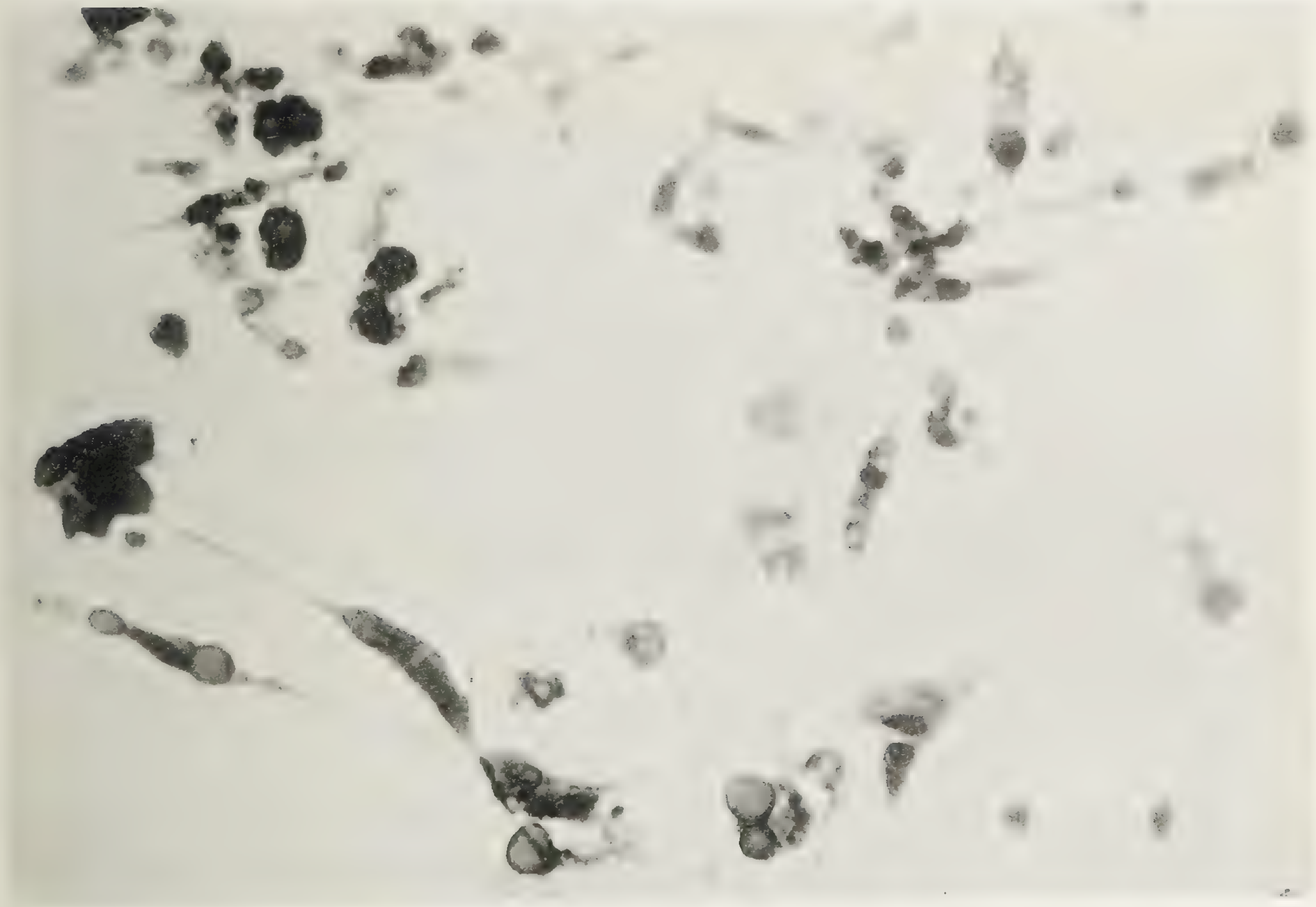

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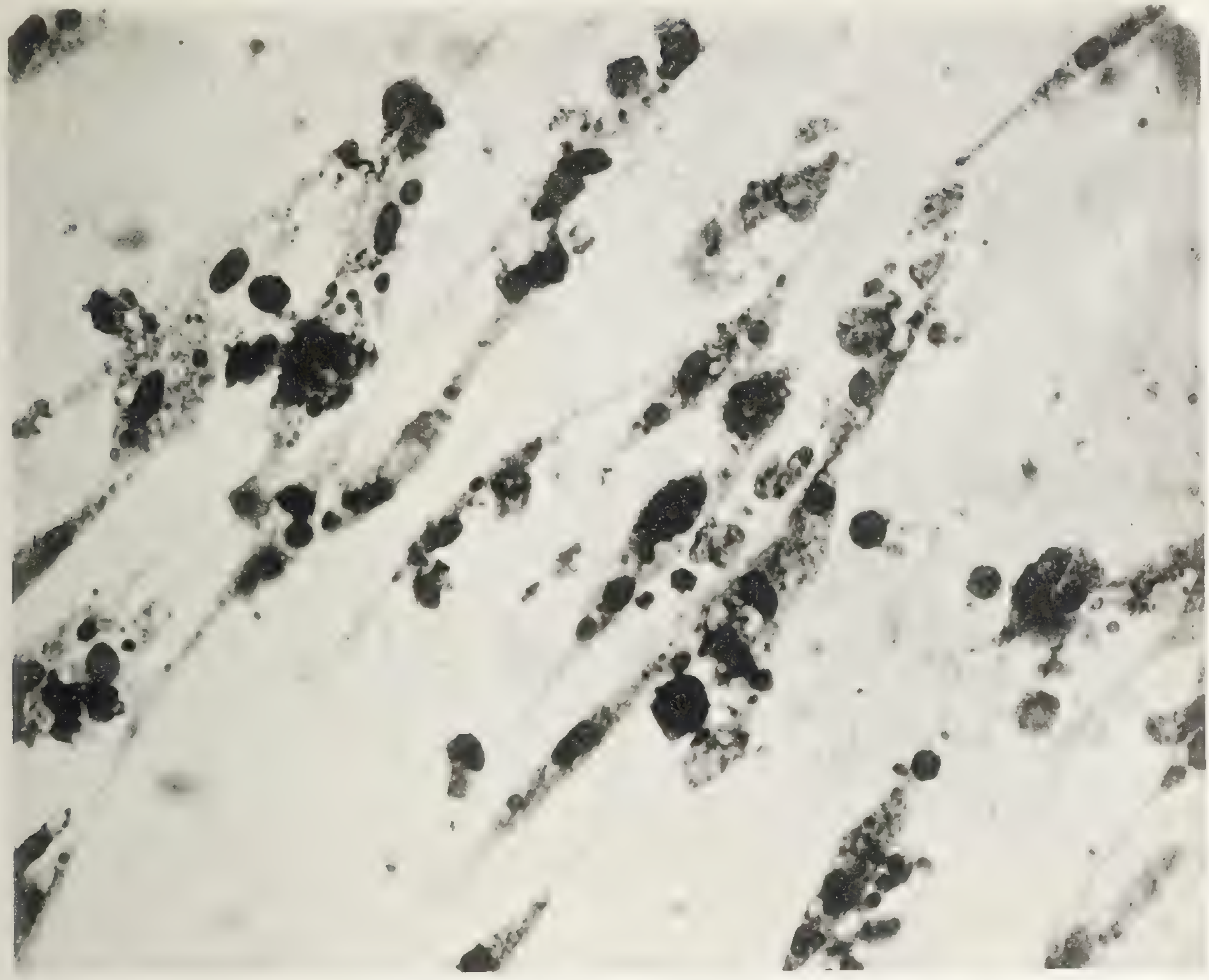

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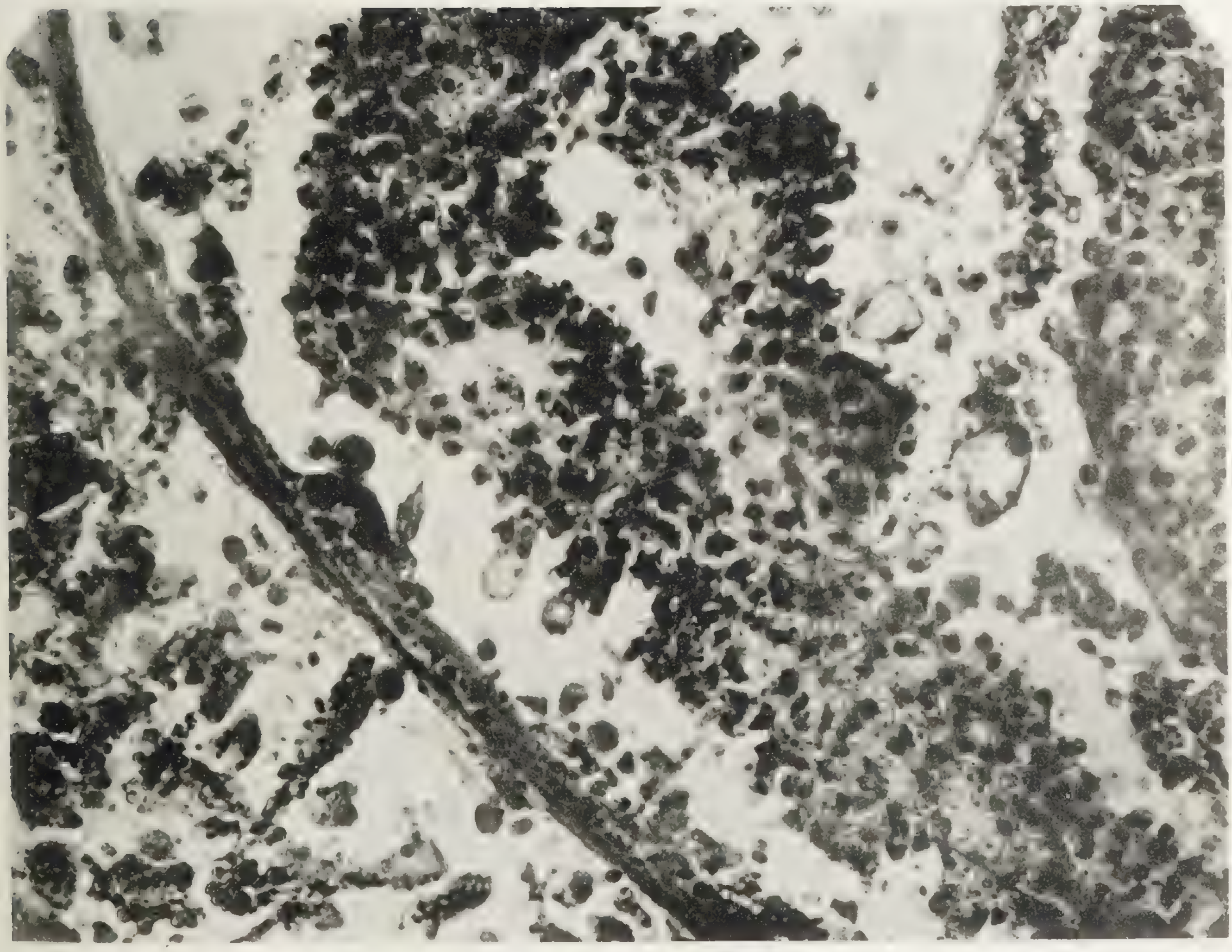

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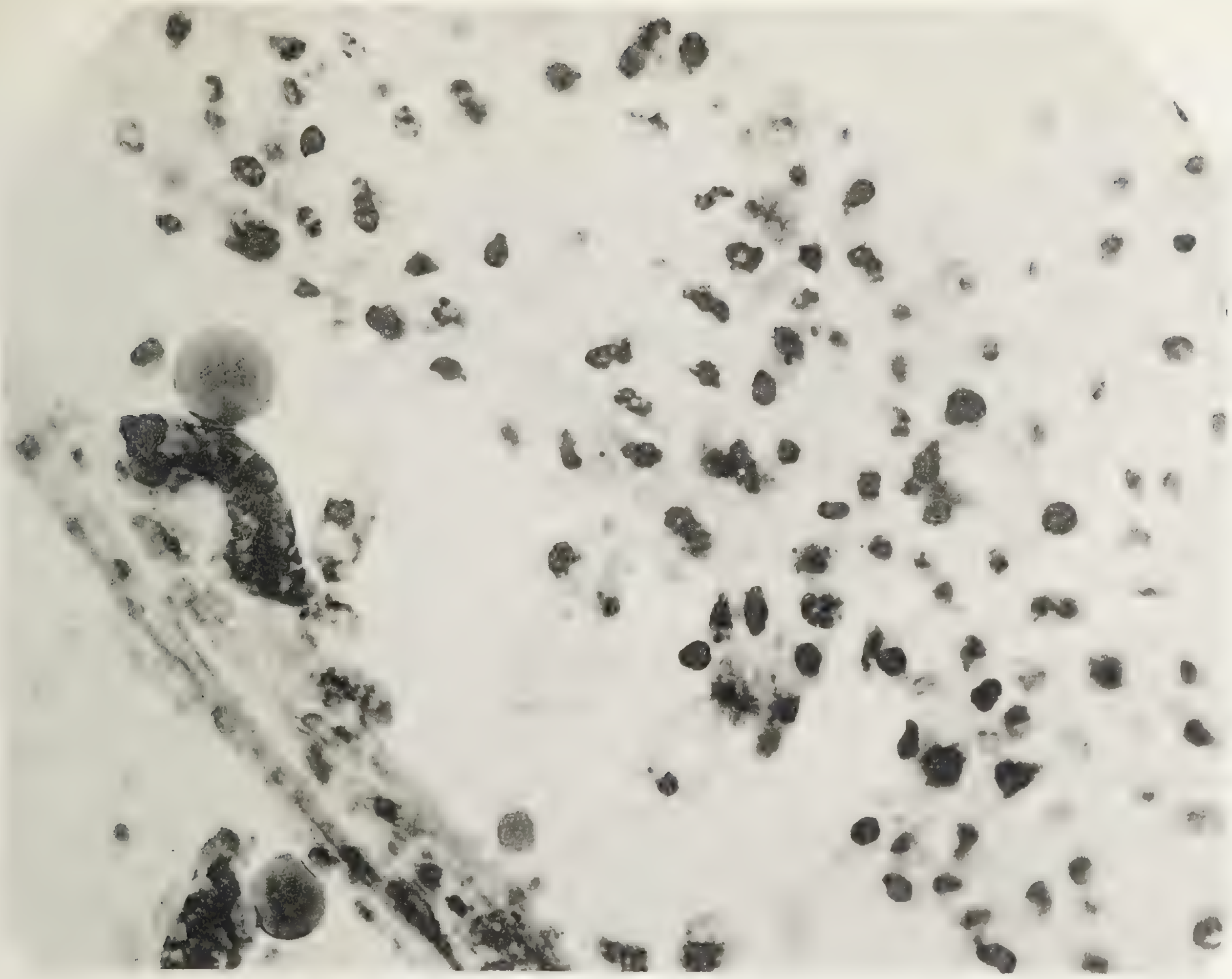

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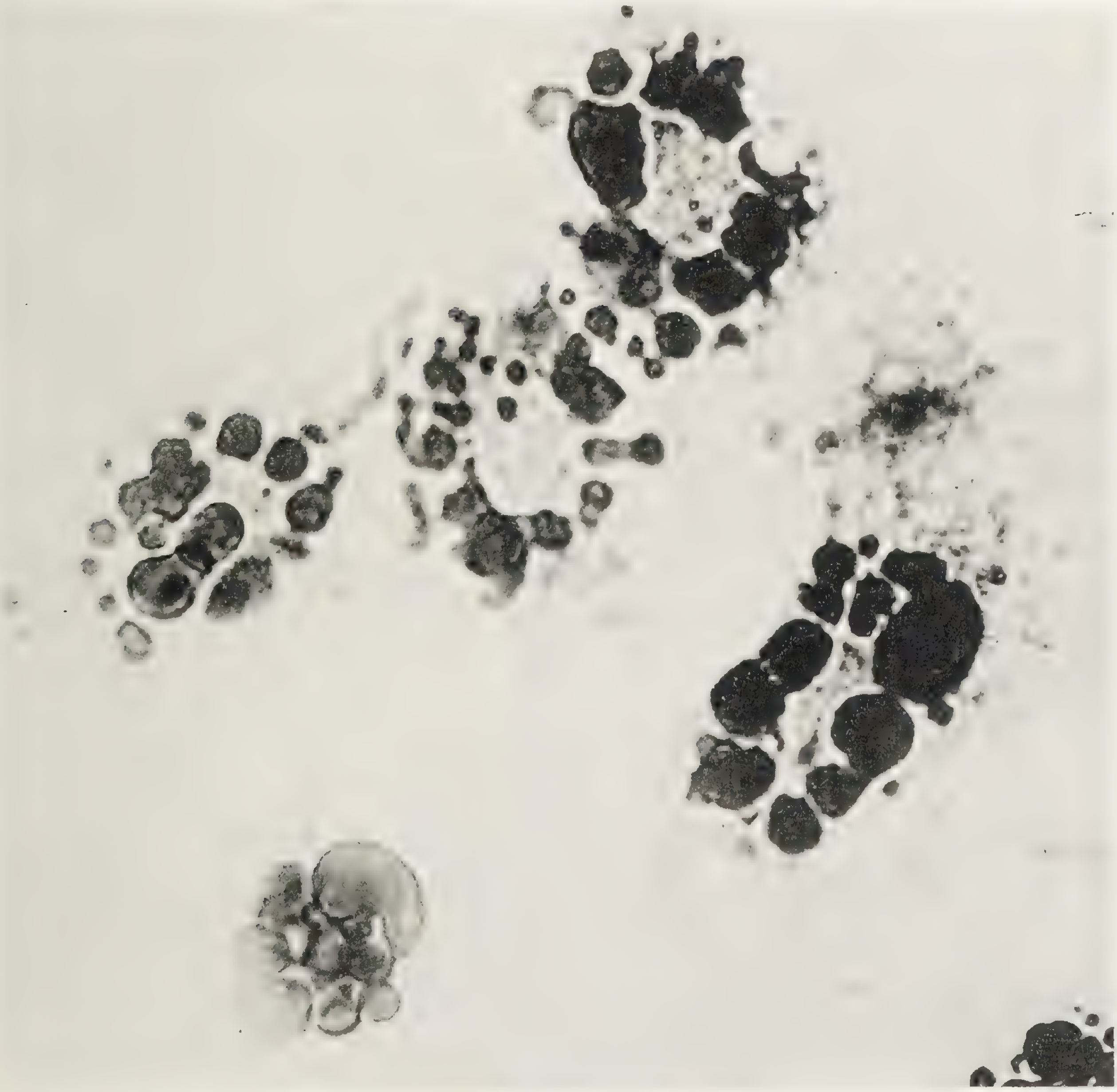

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Weil

Growth of tissue

RAPID FILTRATION OF AGAR AND GELATIN

W. L. HOLMAN

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RAPID FILTRATION OF AGAR AND GELATIN.*

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In the ordinary routine work of a bacteriological laboratory the preparation and filtration of agar and gelatin is probably the most troublesome as well as the most tedious of any technic.

Since Koch revolutionized bacteriological methods, in 1880, by the introduction of the agar-agar culture media, which allowed the satisfactory isolation of organisms, as well as the preparation of stable solid media, workers have been endeavoring to find a more satisfactory and less tedious method of filtration. In many laboratories filtration is carried out by steam or hot water funnels and absorbent cotton, in others the funnel with the absorbent cotton or canton flannel along with the filtering flask is placed in flowing steam. Many do not even go to this trouble and are satisfied with filtering the hot medium through cotton at the room temperature. Drigalski[1] has described "Ein Schnell-filter für Agarlösungen," which is a device for the preparation of agar with the subsequent filtration of the medium in the same apparatus. It gives a large filtering surface and adds thereby to the rapidity of the process.

A method, which has apparently been overlooked, and which is not used in America, as far as the writer knows, was described by

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[1] *Centralbl. f. Bakt., I. Abt.*, Orig., 1906, 41, p. 2.

Bisserie[1] in 1907. The idea involved in his description has been used and modified in our laboratory, and has given us the utmost satisfaction.

After experimenting with many different processes, the simplicity, rapidity, and great efficiency of our modification over other methods has prompted us to bring forward our article. Our procedure in the preparation of 1,000 c.c. of agar is as follows:

A. DOUBLE STRENGTH BROTH.

1. Weigh out—

Peptone (Witte)	10 gm.
Sodium chloride	5 "
Meat extract (Liebig's)	3 "
Add—Distilled water	500 c.c.

2. Mix together and thoroughly boil for half an hour in a double boiler. "As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using 25 per cent solution of common salt, which raises the boiling point 4.5° C." (Stitt, 1910).

3. Titrate and correct reaction. For neutral agar make this double strength broth neutral. For 0.6+ agar, the broth must be 1.2+, or double the acidity desired in the finished medium. The agar solution, as prepared below, is slightly alkaline or neutral.

Boil again after titration to permit the complete precipitation of the phosphates. Retitrate to control result and to make certain of the stability of the acidity.

4. Filter through ordinary white filter paper. Make up to 500 c.c.

B. AGAR SOLUTION.

Agar-agar (for 1.5–2 per cent in finished product)	15–20 gm.
Distilled water	500 c.c.

The agar should be finely divided and partly dissolved in the double boiler. (We have found the stick agar cleaner and better than either the thread or the powdered agar.) Place the agar mass in the steam sterilizer and heat to 120° C. for a few minutes. This will give a perfect solution of the agar in the shortest possible time, being decidedly more rapid than when the agar is added directly to the ordinary broth. Moreover, the complete solution is an absolute necessity to insure the success of any method of filtration. Mix together A and B while both are still hot; boil together for a few minutes. Allow to cool to 55°–60° C. Add the whites of two eggs well beaten in water to the agar preparation, mix throughly, and pour the mass into the filtering apparatus.

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[1] *Ann. de l'Inst. Pasteur*, 1907, 21, p. 235.

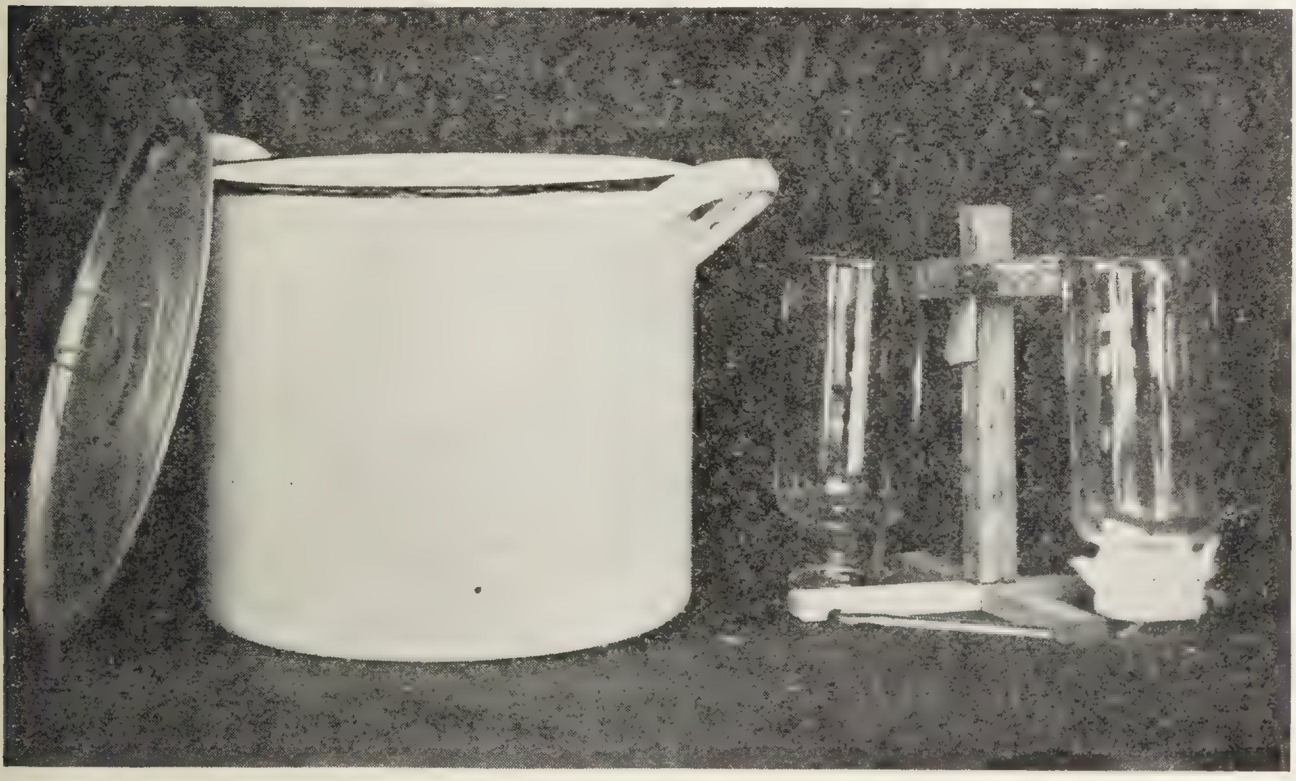

FIG. 1.

FILTERING APPARATUS.

The model as used by us is as follows (see Fig. 1):

1. An enameled pot 8.5 inches high by 8 inches in diameter, with a cover.

2. Four ordinary bottles 7 inches high by $2\frac{1}{2}$ inches wide, with a neck $1\frac{1}{2}$–2 inches in diameter (not wider).

3. Canton flannel cut in convenient sizes to cover mouths of bottles.

4. A wooden appliance to steady the bottles when in position.

5. Small glass rods.

The canton flannel is tied firmly over the necks of the glass bottles. The wooden apparatus is placed in the enameled pot containing the prepared nutrient agar. The bottles are put in position with the covered necks downward resting on the glass rods. The cover is put on the pot and the whole placed in the steam sterilizer, heated to 120° C. for a few minutes, to permit a firm coagulation of the egg, and then allowed to cool slowly.

The principle of this filtering method depends on the expansion of the air in the inverted bottles which, bubbling out during the heating, leaves a vacuum on subsequent cooling, and thus exerts a strong suction on the medium, which is slowly drawn into the bottles. The glass rods prevent the bottles from being drawn tightly to the bottom of the container, which would of course stop entirely all further filtration.

The agar prepared and filtered as above is perfectly clear, much whiter than ordinary agar and there is no clouding on subsequent sterilization. By preparing the agar medium in two distinct stages, so that the agar solution and broth are not repeatedly heated together, we find that the darkening of the medium is avoided.

Gelatin is prepared in the usual manner, and it is found that filtration is accomplished equally well by heating it to only 100° C. in the steam sterilizer sufficiently long for the thorough coagulation of the egg.

Glycerin jelly, for use in the mounting of pathological specimens, which offers exceptional difficulty in preparation, is also readily filtered by this method.

We have purposely excluded all metal from this apparatus, having found that small amounts of iron are sometimes absorbed by the hot media, giving color reactions with certain organisms which is undesirable.

The size of the filtering surface we have found to be of some importance. If the vessel has too wide a neck the filtering cover is drawn so far into the interior as to seriously interfere with the complete filtration. If the area is too small the egg may clog the pores of the flannel.

In using wider mouthed vessels, we have employed a perforated metalic disk over which the filtering cloth is tightly drawn.

Although this is quite satisfactory, as far as the filtration goes, we have, for the above reason, preferred not using metals of any kind in our apparatus.

The advantages of the above method are obvious. The final product, whether agar or gelatin, remains much lighter in color, and is devoid of the fine cloud or haziness so commonly obtained by the ordinary methods of filtration. Moreover, the method may be carried on with greater rapidity and with less personal attention. When the filtration is complete there is remarkably little residue. The simplicity of the method makes it adaptable to every laboratory, without the purchase of a special apparatus.

ON THE METAPLASIA OF BRONCHIAL EPITHELIUM

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BOSTON
MASSACHUSETTS
U.S.A.

ON THE METAPLASIA OF BRONCHIAL EPITHELIUM.*

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During the study of a case of unresolved pneumonia, which contained both areas of organization and abscesses, one field was found which contained two medium-sized bronchi, the mucosa of which was replaced by granulation tissue covered by stratified squamous epithelium. The region from which this block came was then more carefully searched and as many as twelve bronchi were found which showed a like condition. Similar metaplasias of the bronchial epithelium have been recorded from time to time, but this was an unusually good example, on account of its wide extent and owing to the fact that, among the squamous cells, islands of columnar cells remained.

The term "metaplasia" was introduced by Virchow about 1854 and was defined by him as the direct change of one cell into another cell which was architecturally different but which had not completely given up its characteristics or structure.

Schridde holds that this view is untenable, as no facts to support it have been observed either in intra- or extra- uterine life. He designates it "direct metaplasia" as opposed to his own theory of "indirect metaplasia." He believes that the formative cells of the growing layers of any kind of epithelium, or their immediate daughter cells, may give up their specific attributes and revert to a cell which has all the powers of differentiation possessed by the embryonic cells from which the epithelium developed. In other words, such cells may form any type of epithelium, but their power of differentiation is limited to the formation of the epithelial tissues and does not include the transformation into connective tissue or endothelium. Schridde states that by the atypical differentiation of such cells a kind of epithelium

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* Received for publication May 3, 1912.

may be formed which is "heterotype" for the region in which it occurs, and cites as an example the occasional presence of squamous celled plaques in the cardiac end of the stomach.

Lubarsch in general accepts Schridde's view but believes further that completely differentiated cells may undergo the change.

He divides metaplasia into (1) pseudo metaplasia; (2) retrograde differentiation of cells, including Hanseman's anaplasia; and (3) real metaplasia such as is seen in squamous celled tumors of the stomach and prostate.

The author did not have access to the original articles of Lubarsch and Schridde, but by the term "pseudo metaplasia" understands the direct extension of one type of epithelium into a region normally covered by another type, *e.g.*, "ectropion of the cervix," and squamous celled extension to the middle ear after paracentesis. Hansemann's anaplasia is described as a cell change in which there is a loss of a number of chromosomes bringing about an increased power of growth at the expense of cell function.

Ribbert is opposed to the whole idea of metaplasia and explains the changes as coming from: (*a*) cells which have been actually misplaced during embryonic life; (*b*) cells which may appear normal for the region in which they occur but which possess embryonic potentialities; and (*c*) cells which are only partly differentiated and which undergo changes more readily. He thinks that a certain combination of circumstances must also be present before such a change can occur, and for this influence lays stress upon the effects of surrounding supportive tissues. He further points out that newly developed cells are never as highly specialized as the preëxisting cells.

Many other views have been brought forward, but the principal points of the discussion are covered by these four observers.

In discussing the case in hand a few points concerning the normal histology of the part may well be reviewed. Kölliker describes three layers of epithelial cells in bronchi large enough to possess cartilaginous plaques; an outer layer of ciliated and goblet cells, a middle layer of somewhat spindle-shaped replacing cells, and an inner layer of pyramidal formative cells, which rests upon a so-called hyaline membrana propria or basement membrane. Bronchi of less than one millimeter diameter have only a single layer of cells and a basement membrane difficult of demonstration.

The bronchial lesions in the case we are considering were varied. Some sections contained large inflamed bronchi with granulation tissue in the mucosa and a complete denudation of all types of epithelium. The lumina were filled with leucocytes and cellular débris. Other sections showed a thick unbroken stratified squamous epithelium lining the bronchi. These epithelial membranes were usually slightly raised from the granulations by a serous exudate beneath them. Small nests of ciliated columnar cells remained in some of the crypt-like depressions of the mucosa in areas where most of the surface had a squamous cell covering. At the points of the junction of the two types of cells the squamous layers seemed to be continuous with the formative layers of the columnar cell portions.

In still other areas typical ciliated columnar cells were found resting on top of a double layer of low cuboidal cells and appeared to be almost ready to desquamate. If this desquamation had occurred no trace of columnar cells would have remained on the surface. In none of the involved bronchi were the basement membranes demonstrable.

In the lung tissue proper there were signs of a rather prolonged inflammation; larger and smaller abscesses were present; the fibrin had the appearance of being old and hyaline, and in many places actual organization had taken place. Some of the alveoli were free from fibrin and instead contained large amounts of serous fluid and many pigment-bearing endothelial cells. The alveolar walls were thickened and the pleura was much fibrosed.

In seeking an explanation for the change from columnar to squamous cells each layer mentioned above must be considered.

First, the ciliated and goblet cell layer was found remaining only in the most protected depressions and was never seen to be in any way connected with the changed cells. On the contrary, where it was not already destroyed, it was being thrown off into the lumen. From this it seemed that there was no evidence that the direct metaplasia of Virchow, the change of the columnar ciliated to the squamous cell, had occurred.

In the " replacing layer " one could not feel so certain, for no cells resembling them were found. It was probable that they had either desquamated directly or had become ciliated and then desquamated. The extensive granulations in the mucosa would point to a wide desquamation of epithelium having taken place. The regeneration must have come, then, from certain remaining cells of the growing layer, and it is to these cells that we looked for evidence of the changed progeny. These cells themselves showed little further than that they were more polygonal and less pyramidal than normal. But their environment was changed, for the basement membrane had disappeared and they rested directly upon granulation tissue. This fact seemed very important, for partial desquamation followed by complete regeneration we believe is a common occurrence in acute inflammation of the bronchi.

In diphtheria, fibrin casts including the epithelial lining of the bronchi may be coughed up, and here Mallory has pointed out the infrequency with which one sees the diphtheritic exudate extending below the membrana propria. Its action is believed to be that of a limiting membrane which allows the separation of the cast to take place. It also serves as a favorable surface for the regeneration of the normal columnar cells.

Three other cases of metaplasia of bronchial epithelium were studied. One was a case of broncho-pneumonia with abscesses. Instances were observed where bronchi, whose walls were partially destroyed by abscesses, retained an uninjured basement membrane in other less severely attacked parts. In one bronchus, where the membrane was broken only at one point, there was present at that point an island of squamous cells. In the second case a large secondary growth of sarcomatous nature had injured many bronchi and partial regeneration had occurred. Stratified squamous epithelium had replaced the normal columnar cells in some of the bronchi, and definite basement membranes were not demonstrable.

The third case was a primary carcinoma of the lung arising in the bronchi. Here the normal relations were completely lost. The lower layer of cells remained columnar in shape, while the more superficial layers were of typical stratified squamous cells.

In none of the cases was there any example of stratified squamous epithelium resting on a well developed basement membrane.

Difficulty was experienced in demonstrating a definite basement membrane in some apparently normal bronchi from other cases, and the membranes when present did not always react equally toward the special stains generally recommended for demonstrating them. Their histological appearance and chemical nature did not seem to be constant for bronchi of any given size. This latter observation is mentioned as an important obstacle in the way of proving that the destruction or absence of the membrana propria is the essential factor in epithelial metaplasia of the bronchi. A list of stains used is given in the order of their efficiency: Mallory's phosphotungstic acid hematoxylin; Pianese's picronigrosin; Van Giesen's connective tissue; Mallory's analine blue; hematoxylin-eosin; eosin methylene blue. The membrana propria did not react to Verhoeff's or Weigert's elastic stains as stated in some texts.

In connection with the study of these cases, Dr. Klotz called my attention to a good example of metaplasia of the stratified squamous type in the pancreatic ducts. The ducts were dilated and some contained calculi, while others were almost completely filled with the proliferated squamous cells. The remainder of the pancreas showed rather advanced chronic interstitial pancreatitis. In the affected ducts the walls were actually inflamed. Many polynuclear leucocytes were present. As the basement membrane of the pancreatic duct is not comparable to that of the lung, the case, while interesting, does not bear directly on the relation, if indeed any exists, between metaplasia and the presence or absence of the membrana propria.

CONCLUSIONS.

1. The evidence concerning metaplasia, as observed in this case, is strongly against the theory of the " direct metaplasia " of Virchow.

2. Our findings, in a measure, agree with Schridde's idea in so far as they show that the metaplastic cells are newly formed cells and that they come from the growing layer.

3. They support Ribbert's views in that the new-formed cells are less highly specialized than the normal cells, and also that the surrounding tissue changes have a strong influence on the type of the new cells formed.

4. They seem to contradict the necessity of the presence of embryonic rests, as they could hardly have been so numerous as simultaneously to set up the process in several different bronchi.

5. Another idea which is brought forward is that the bronchial mucosa destroyed beyond the hope of specific regeneration seeks to protect itself with the best reparative cells it can produce in its exhaustive state.

6. Assuming that there is a histo-chemical identity of the so-called basement membrane, it suggests that there is a link between the injury to these membranes and tumor growth; that is, that the irritants and inflammations which destroy them open the way for metaplasia and new growth.

[Thanks are due Dr. Klotz for his valuable suggestions during the preparation of this paper.]

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DESCRIPTION OF PLATES.

PLATE XXX.

FIG. 1. — Squamous cell metaplasia of bronchial epithelium. L.P.

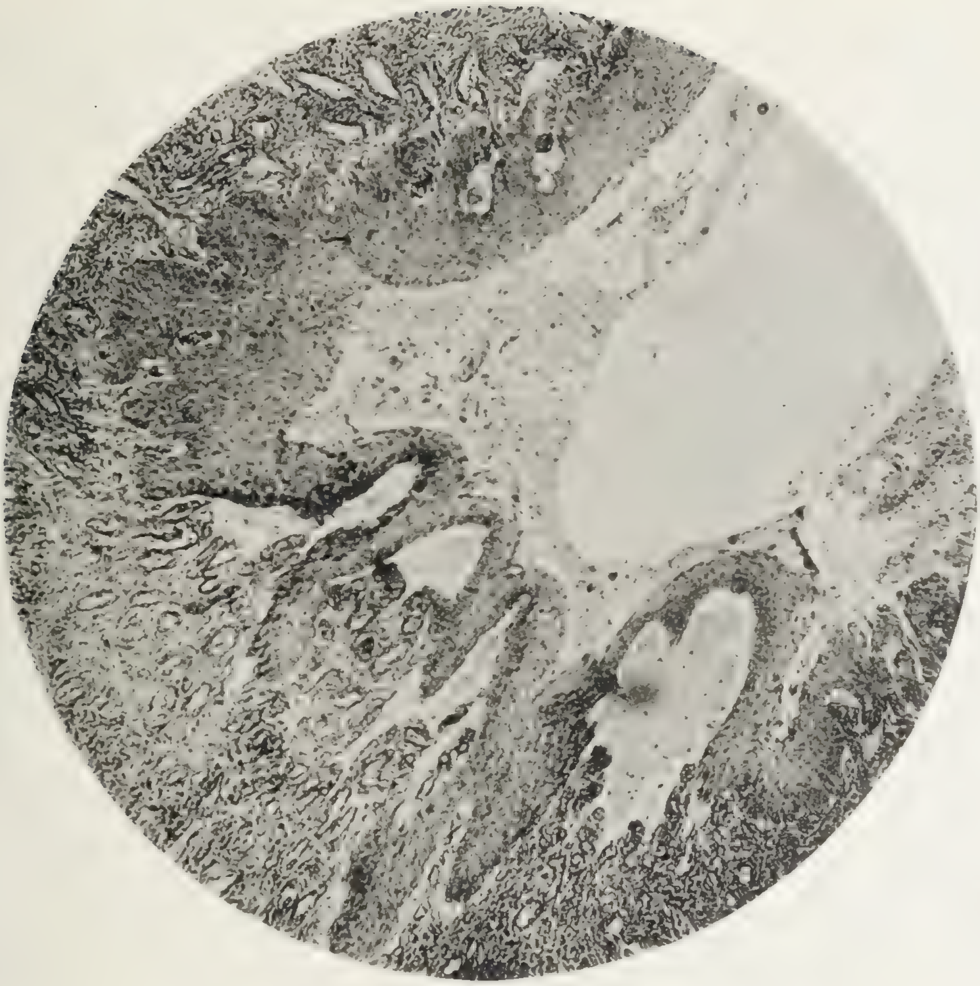
FIG. 2 — Squamous cell metaplasia of one bronchus, columnar cells remaining in adjacent bronchial epithelium. L.P.

PLATE XXXI.

FIG. 3. — Squamous cell metaplasia of bronchial epithelium showing a crypt covered with columnar cells. The basal layer is continuous for both columnar and squamous types. H.P.

FIG. 4. — Squamous cell metaplasia of bronchial epithelium. Crypt showing a layer of columnar cells resting on a double layer of cuboidal cells. Mass of cells to the right of squamous type.

THE JOURNAL OF MEDICAL RESEARCH, Vol. XXVI., No. 3, July, 1912.



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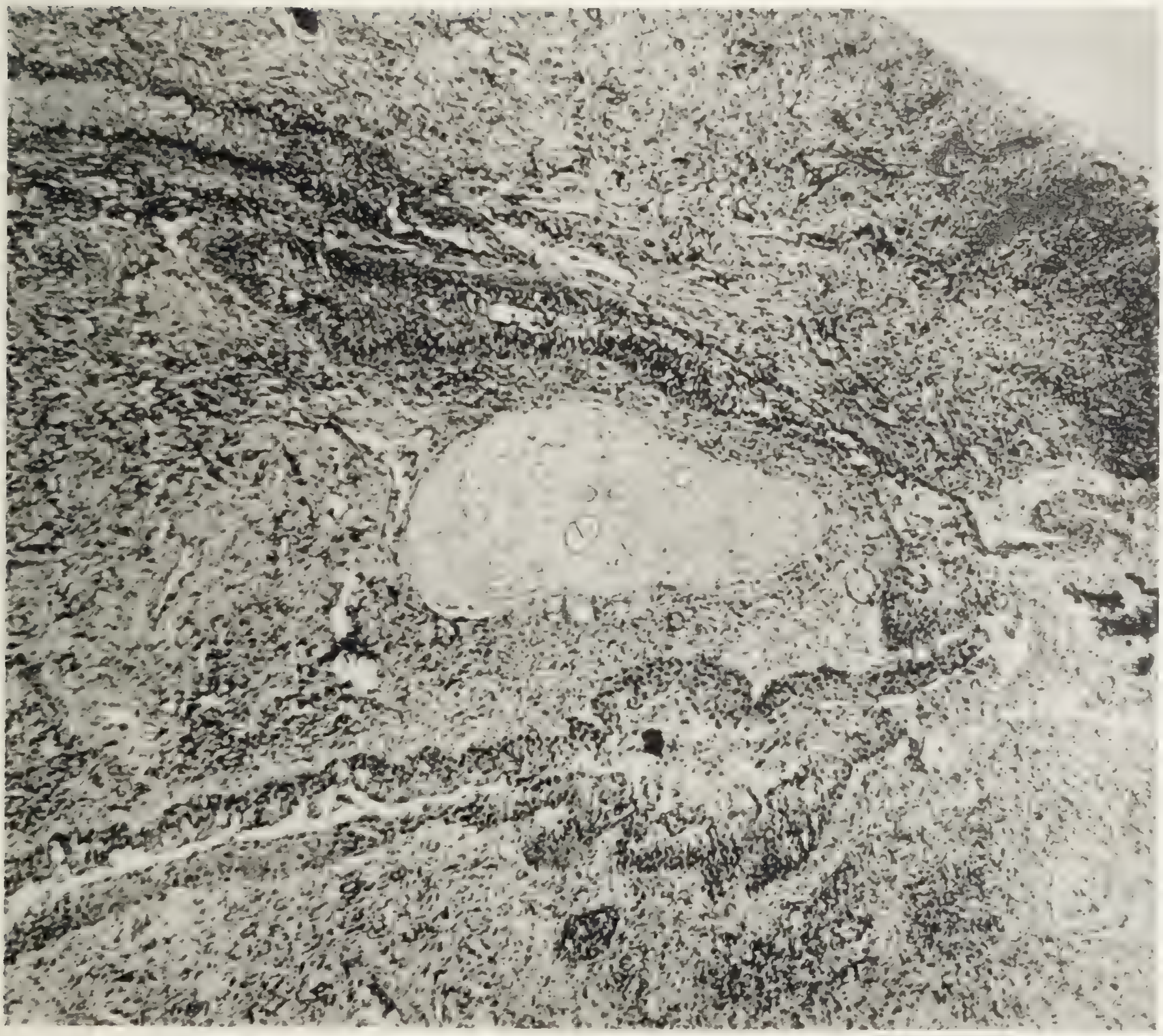

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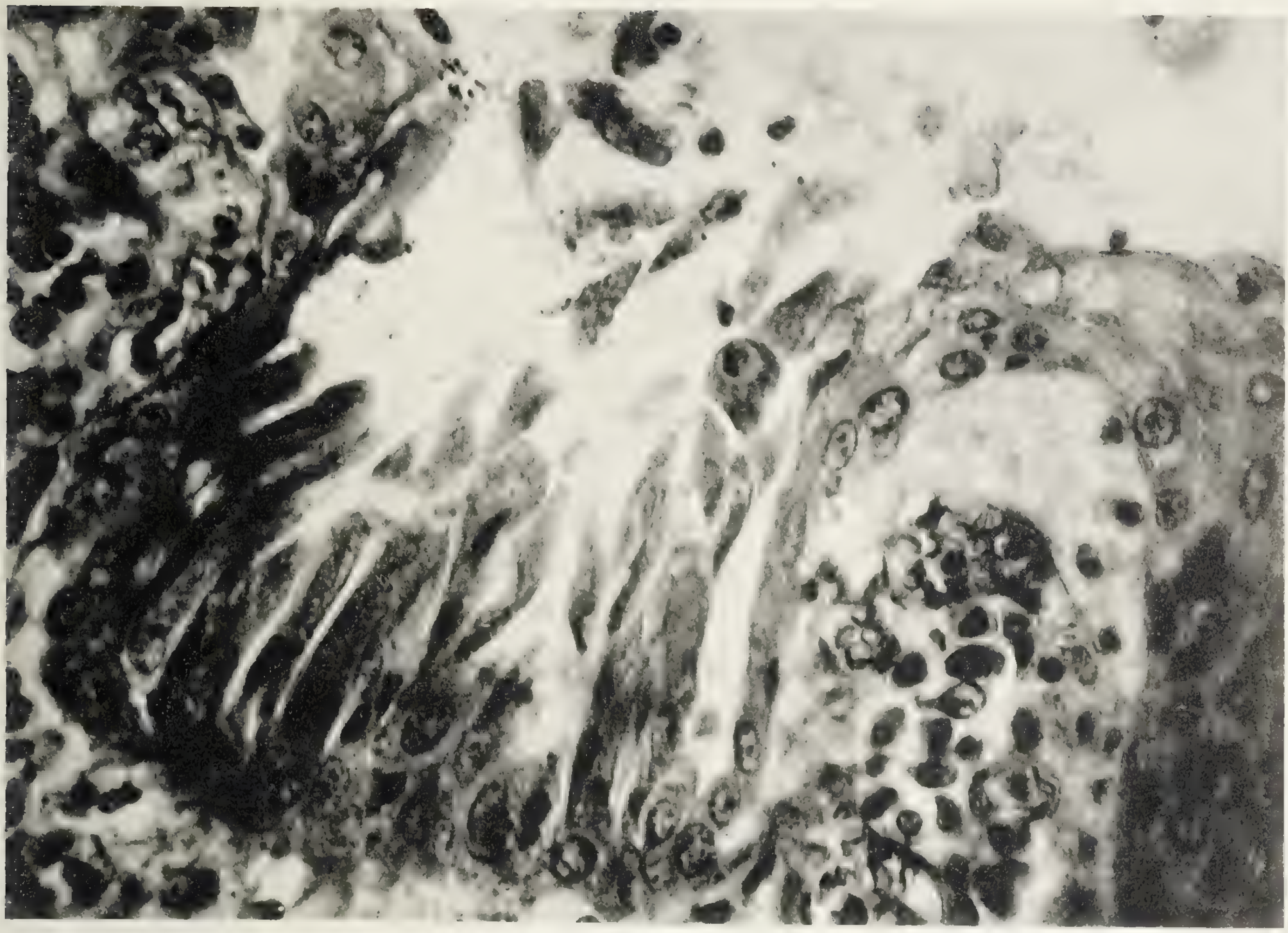

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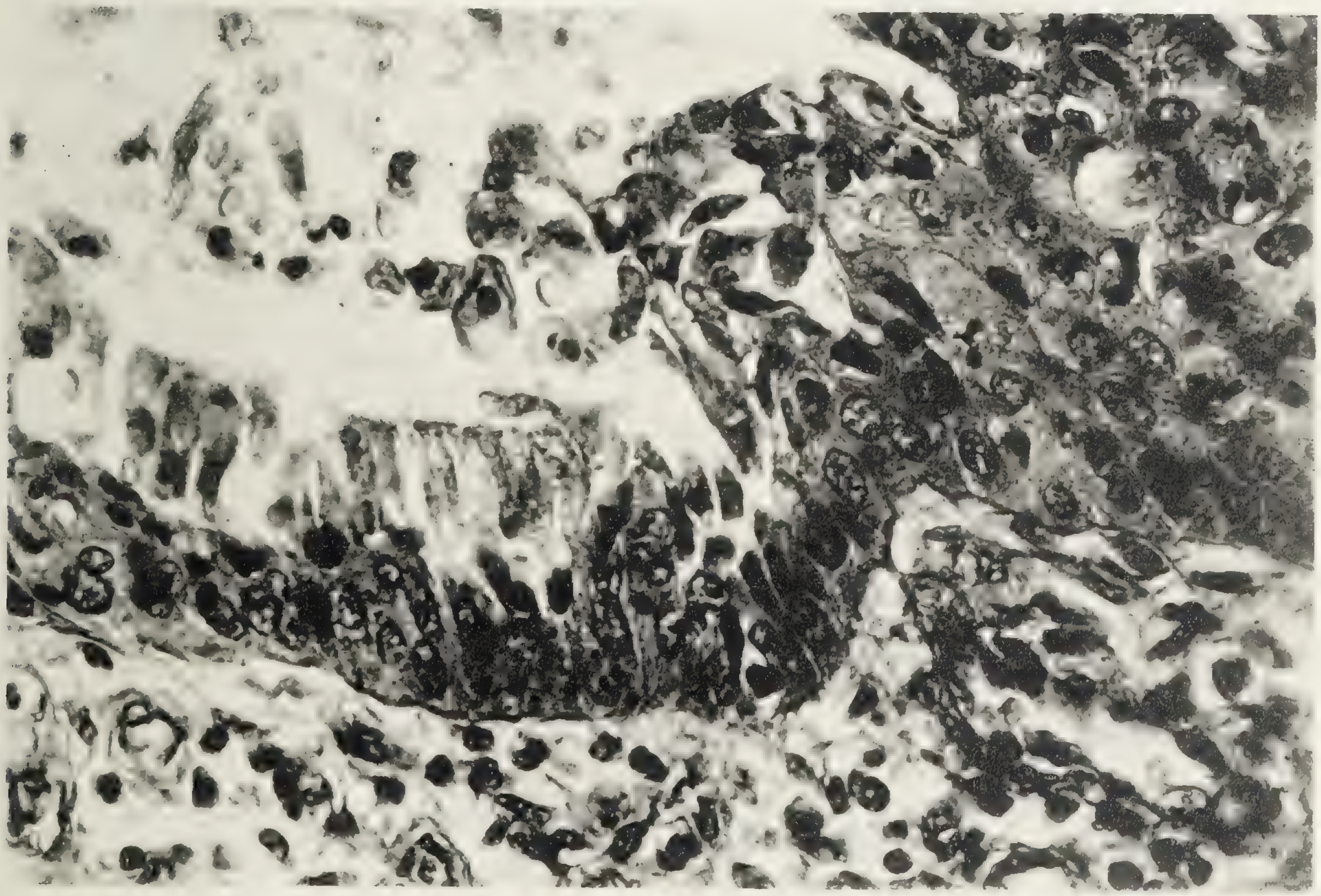

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PRIMARY CANCER OF THE LUNG

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(Magee Pathological Laboratory, Mercy Hospital, Pittsburgh, Pa.)

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BOSTON
MASSACHUSETTS
U.S.A.

PRIMARY CANCER OF THE LUNG.*

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Primary carcinoma of the lungs is a rare affection. Its incidence may be roughly indicated by the statistics of Reinhardt, Fuchs and Passler. Reinhardt,[1] in eight thousand seven hundred and sixteen autopsies at the Dresden City Hospital, found five hundred and forty-five cases of carcinoma, of which five were primary in the lungs. Fuchs[2] found in the Munich records eight cases of primary lung cancer in twelve thousand three hundred and seven autopsies. Passler[3] records, out of one thousand cases of malignant disease from the Breslau Pathological Institute, four primary sarcomas and sixteen primary carcinomas of the lung. It must be borne in mind that it is difficult to differentiate between primary carcinomas arising in the lung and those of bronchial origin, and that probably many tumors of the latter type are included in the statistics quoted above. Rolleston and Trevor[4] conclude that these figures for lung cancers are too high, and state that there is no reason for believing that carcinoma is more frequent than sarcoma. Out of three thousand nine hundred and eighty-three autopsies at St. George's Hospital they found three sarcomas and no carcinomas primary in the lungs.

While there have been a large number of cases reported, not all have been undoubted cases of primary cancer of the lung, and in many cases the reports were incomplete. From a critical examination of the literature up to 1896 Passler[3] gathered the data of seventy cases in which the reports were complete enough to be of value, and to these added four from the Breslau Pathological Institute. From a partial review of the literature since that time I have been able to collect the reports of ten additional cases which, with the one here reported, makes a total of eighty-five from which statistical data concerning the type of tumor can be drawn. The

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ten cases were reported by Ernst,[5] Ribbert,[6] Delorme,[7] Bremken,[8] Ennet,[9] Herrmann,[10] Parrow,[11] and Kaminski[12] (one case each), and Lammerhirt[13] (two cases). The last five were from the Greifswald Pathological Institute.

Of these eighty-five cases many are probably and some certainly of bronchial origin, but no attempt has been made to separate them. In the following table these cases have been classified according to the histological type of tumor present.

Squamous celled	12
Cylindrical celled	24
Cubical celled	1
Transitional cells	1
Polygonal cells	9
Polymorphous cells	11
" Large epithelial cells "	4
Scirrhous	3
Cancer, but the type not stated	12
No microscopic report	8

Among the squamous celled tumors is included one in which it was stated that the cells were " similar to alveolar cells." Four of these tumors showed keratinization. Of the cylindrical celled cancers three are described as forming gland structures. Several of the polygonal and polymorphous celled growths are stated to have shown in places cylindrical or squamous cells, but a more indefinite type of cell predominated.

As to the etiology of lung cancers but little more can be said than for the etiology of malignant growths in general. The case reported by Ribbert[6] was associated with fibrosis of the lung, together with other signs of a previous inflammatory process, and he calls attention here to his well-known theory of the origin of cancer, which is apparently supported by this instance. The cases of Grant,[14] Lawrence and Suckling,[15] Friedlander,[16] and Ennet[9] were associated with pulmonary tuberculosis. It has been stated by Wieber[18] and restated by Osler that cancer of the lung is remarkably frequent among the cobalt miners of Schneeberg. It is possible that here a type of pneumonokoniosis is a factor in the etiology. The case reported in this paper was associated with marked anthracosis. But both tuberculosis and anthracosis are so common, and cancers of the lung so rare, that an etiological relationship could hardly be considered.

It might be remarked, concerning the reported cases of cancer occurring at Schneeberg, that definite histological reports of these tumors are not forthcoming and that some investigators suggest that mediastinal tumors or even cases of Hodgkin's disease have been included in the series.[19]

There are three possible points of origin for the tumors of the lung; the alveolar epithelium of the air sacs, the bronchial epithelium and the epithelial lining of the bronchial mucous glands. Concerning the latter but little can be said and it may possibly be the point of origin of some of the apparent adeno-carcinomata. About the other two, however, considerable polemical literature has accumulated. Some of the tumors mentioned above have undoubtedly arisen from the bronchial mucosa remaining isolated in the bronchi. Others, by their anatomical situation, and the fact that they have infiltrated the lung along the bronchial tree, have left but little doubt as to their bronchial origin. But the majority, on account of their large size or wide dissemination, offer nothing in the gross appearance to indicate from what structures they have arisen.

As to the cylindrical celled tumors, it is generally conceded that they arise from the epithelium of the bronchial mucosa, or possibly from the bronchial mucous glands. The fact is to be considered, however, that the embryonic type of alveolar epithelium is cylindrical in character. And the possibility also presents itself that in atelectatic lung tissue the epithelium may undergo a true metaplasia to cylindrical epithelium and, having been rendered functionless, acquires the " habit of growth," and produce a tumor.

The origin of the squamous celled tumors is still more difficult to explain. Some of them have been of undoubted bronchial origin, but in others this is not so clear. Siegal, Perls, Grunwald and Wechselmann believe that they arise from the alveolar epithelium. Tillmann speaks of a tumor composed of cells "similar to normal alveolar epithelium," and to these tumors Grunwald has applied the term "alveolar epithelioma." But it is a far cry from simple alveolar epithelium to a structure composed of stratified squamous cells showing intercellular bridges, forming types of epithelial pearls, and in some cases producing keratin. On the other hand, to explain an origin from the bronchial mucosa there must be called into play a process of metaplasia. It is interesting in this connection to note that such a process has been noted in the bronchi, in the absence of any malignant change, by Friedlander, Curt Wolf, and recently by S. R. Haythorn. The latter observer found areas of metaplasia to squamous epithelium in the bronchial mucosæ of three lungs from this laboratory, all being cases of pneumonia. He also found, in sections from the lung described below, areas of metaplasia in the bronchi, the columnar cells being replaced by squamous cells similar to those forming the tumor. Haythorn does not consider that there is a direct metaplasia of the columnar cells to squamous cells, but that the latter arise from the deeper epithelial cells after the columnar cells have been destroyed or lost during an inflammatory process. On account of these observations it is probable that the metaplasia is the primary process, and that the tumors arise from such metaplastic epithelium. This is the explanation which Ernst[5] accepts for his case.

A third possibility may be briefly mentioned, that some of those tumors are in reality endotheliomata. This is the view of Schottelius.

The intermediate types of cancer offer but little more in their microscopic structure than they do in the gross appearance towards solving the question of their origin. We must agree with Ribbert,[6] that "it is impossible, in cases of far-advanced carcinoma, to determine anything of their origin."

The case here reported is one of non-keratinizing squamous celled carcinoma arising in and remaining isolated to the right lung. The patient, Mr. M. C., aged sixty-two, was admitted to the service of Dr. C. O. Goulding in the Mercy Hospital on Aug. 1, 1911, in an intoxicated condition. The family history was not important. He had gone through the usual diseases of childhood, and had repeatedly been ill following intoxication. The patient had been drinking heavily for some days before admission. The day following his admission his temperature rose to 102°. On examination the right chest was found on percussion to be flat from the clavicle to the fourth rib anteriorly, with bronchial breathing over this area. He was coughing and expectorated blood-tinged sputum. For a week the temperature varied between 98° and 101°, being low in the morning and high in the evening. At the end of a week râles appeared at the right apex. The temperature gradually subsided, but varied from 97° to 100° for his remaining illness. The consolidated area never became resonant. The patient grew very weak and developed a marked pallor, but never appeared cachectic. For two weeks before death he was delirious, and had no control of his sphincters. Sputum examinations from time to time revealed the presence of pneumococci and streptococci, tubercle bacilli were not found, and cancer tissue, if present, could not be recognized. Death occurred on September 4. A clinical diagnosis of unresolved pneumonia of the right upper lobe was made.

At autopsy a small portion of the right lung was found to be adherent to the sternum. In the left pleural cavity there were many old adhesions, and a small quantity of blood-stained fluid. The upper and middle lobes of the right lung were firmly adherent to the parietal pleura and the pericardium. The left lung showed a marked marbling of the pleura due to advanced anthracosis. On section the upper lobe was seen to be rather moist and crepitated throughout. The lower lobe contained a moderate amount of grayish fluid. The bronchi contained a mucoid exudate. Over the upper and middle lobes of the right lung there was a dense, plate-like organized exudate. Over the upper third of the lower lobe there was a fine, sand-like fibrinous exudate. The anterior surface at the lower two-thirds was free from exudate. On the posterior surface there was a thin, very vascular membrane. The upper and middle lobes and the upper part of the lower lobe were completely solidified. The lower lobe crepitated. On section the upper lobe was seen to be replaced by an enormous mass composed of a pinkish, cream-colored tissue of a pasty consistency. Posteriorly this mass was bounded only by the thickened pleura and about two centimeters of blackened atelectatic lung.

In two places the mass extended forwards to the pleura. The cut surface of the tumor measured 9.5 by 6 centimeters. In the middle lobe there were two smaller areas of similar, soft friable tissue. The lung tissue of the lower lobe was of a grayish red color; the lower border was edematous. The only remaining normal tissue in the areas of new growth were some bands of connective tissue and some blood vessels. In the main bronchus of the upper lobe there were soft papillary projections into the lumen. The peribronchial lymph nodes of both lungs showed marked anthracosis, but no necrosis or evidence of new growth. One gland at the bifurcation of the trachæ was calcified.

The other autopsy findings were of minor interest. The aortic and mitral valves were sclerosed; there was sclerosis of the aorta and coronaries. There were multiple hemorrhages into the spleen and the submucosa of both the large and small bowels. The left lateral sinus contained a firm, white thrombus, which was adherent. There was a chronic inflammation of both middle ears. The testicles were fibrosed. There were no new growths found in any part of the body, save the right lung.

The microscopical examination of the tumor showed it to be composed of cell masses having a general arrangement suggestive of an alveolar structure Many of these masses were quite solid, but the majority of them contained an open space in the center filled with necrotic material. Here and there large, irregular arborescent or serpentine masses of tumor cells were formed, surrounded by a necrotic material. The stroma was thin and the relationship of the tumor cells to it was quite definite. Lying upon the basement membrane of the stroma there was a single layer of columnar cells, but the layer above this consisted of smaller, round and irregular cells, and above this layer the cells were squamous in type. As a rule the tumor cells formed only a thin layer, ten or fifteen cells deep. The upper ones were definitely squamous in type, showing intercellular bridges, and forming here and there small concentric whorls, which seldom consisted of more than five or six elements. No keratinization could be demonstrated, although a large number of sections were examined, using not only the usual stains but also Gram's, which, as Ernst[5] has pointed out, is a selective stain for keratin. The cells had large, deeply staining nuclei, and a rather indistinct, faintly staining protoplasm. Mitotic figures were numerous.

As has been mentioned, the stroma was very delicate, consisting of a few fibrils supporting the cell masses. Sections stained with Van Gieson's and Mallory's stains showed the greater part of the stroma to be composed of white fibrous tissue, but sections stained by Weigert's elastic tissue showed the presence of fibers of the latter type also. This fact, together with the alveolar structure of the tumor masses which became more apparent in these sections, suggested that the original stroma of the lung had been retained, and that the tumor had grown into the lung along the alveolar walls. But the alveolar structures were usually larger than lung alveoli, and they were so indefinite and inconstant as to preclude the possibility of drawing any definite conclusions of their origin. The open spaces in the center of the masses, however, were clearly not due to necrosis of the tumor, as the cells bordering them did not show signs of destruction.

The stroma carried only a few small capillaries and, when compared with the amount of tissue supplied, appeared quite inadequate. The necrotic portions of the tumor showed the presence of a number of polymorphonuclear leucocytes and here and there an infiltration of lymphocytes into the stroma. About the tumor there was seen a border of compressed and fibrosed lung tissue, showing a considerable infiltration of lymphoid cells. A moderate amount of anthracosis was present.

Sections of the nodules in the bronchi showed an invasion of the bronchial wall by tumor from the surrounding tissue. Sections of the peribronchial lymph glands failed to show any evidence of metastases.

There are several points about the case that merit discussion. Definite conclusions as to the origin of the tumor cannot be drawn from gross appearances on account of the wide involvement of the growth obliterating all structures. In the microscopic examination of the specimen there is only one point which is helpful; namely, the finding of areas of metaplasia of columnar to squamous epithelium in the bronchi, unassociated, however, with any malignant change. It is quite probable that the tumor arose from epithelium of this nature.

The absence of keratinization is of interest. Although Ernst[5] lays especial stress on the formation of keratin in this type of lung tumor as essential to the diagnosis, it is to be noted that this process occurred in only four of the twelve squamous celled cancers tabulated above. Moreover, non-keratinizing squamous celled cancers are frequently noted in other organs as, for instance, in the cervix uteri and the bladder. In the case here reported the presence of definitely squamous cells with whorl formation and the presence of intercellular bridges leaves but little doubt as to the diagnosis.

The apparent alveolar structure, as though the tumor had invaded the lung parenchyma along the alveolar walls, seems to occur frequently in carcinomata of this organ. In eight of the eighty-five cases mentioned above such an arrangement was mentioned as being present. Passler observed in his third case that "the cancer fills the original alveoli and the alveolar septa form the stroma." The finding of elastic tissue fibers in the stroma of our tumor is additional evidence that such a process has also occurred here. Siegert,[17] too, considers that cancers invade the lung in this manner and Ribbert[6] agrees with him, although the latter author points out that there is another mode of invasion; namely, along the lymphatics of the alveolar septa.

Finally, it is noteworthy that so large and so cellular a tumor could be produced without forming distant metastases, even in the regional lymph nodes.

CONCLUSIONS.

1. Primary carcinoma of the lung is a rare affection, occurring approximately once in sixteen hundred autopsies.

2. Probably the majority of so-called cancers of the lung are in reality of bronchial origin.

3. Squamous celled cancers of the lung probably arise in the majority of cases from bronchial epithelium which has undergone a metaplasia.

4. Some of these tumors apparently invade the lung along the alveolar wall, retaining the alveolar septa as stroma.

[I here wish to express thanks to Dr. Oskar Klotz for his criticisms and aid in preparing this paper, and to Dr. S. R. Haythorn for the photomicrographs.]

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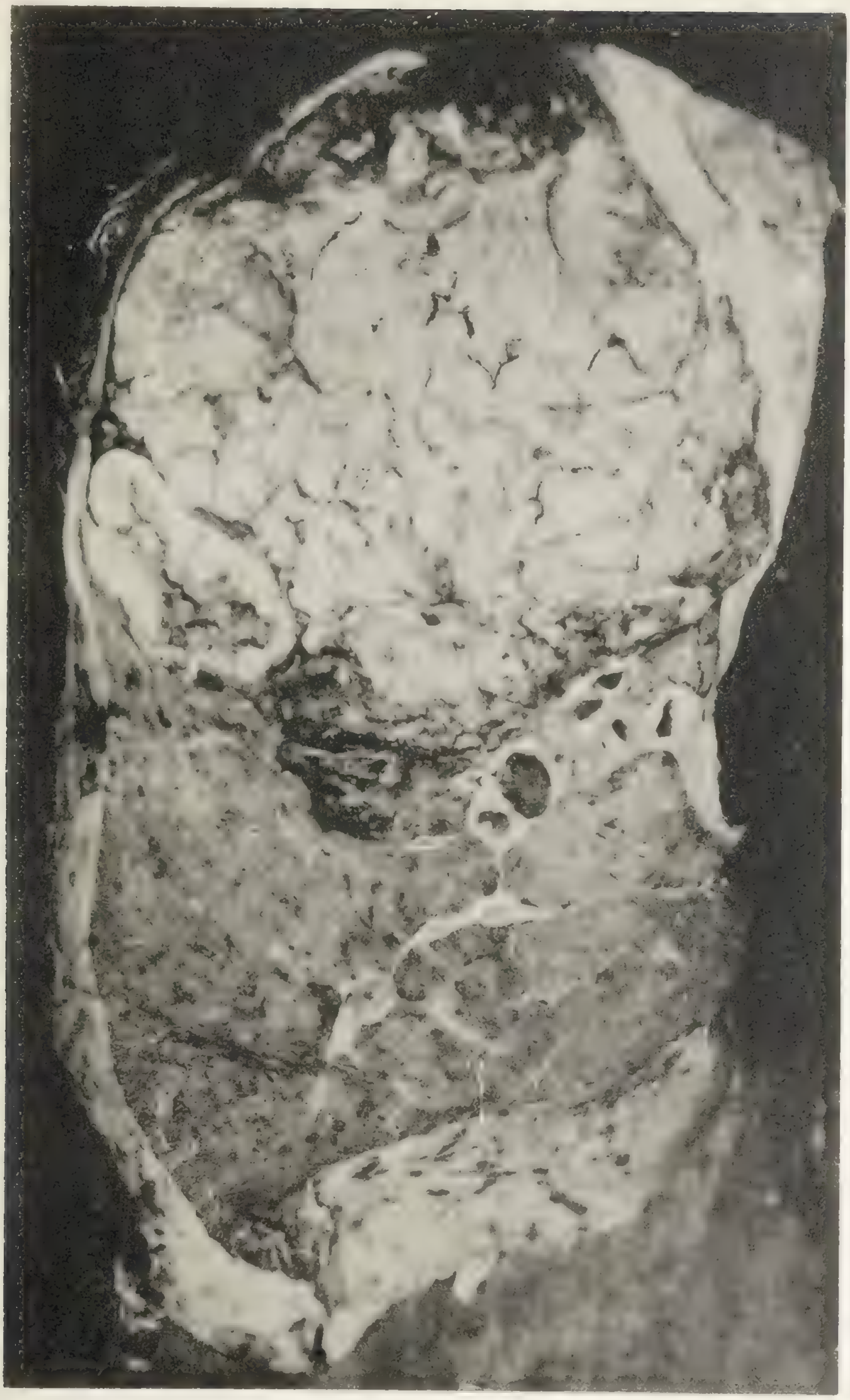
DESCRIPTION OF PLATE XVII.

Fig. 1. — Primary cancer of right lung.

Fig. 2. — Section at margin of cancer of lung, showing alveolar character with central lumen.

Fig. 3. — Cancer of lung showing squamous character of tumor cells, and apparent " perivascular " arrangement.



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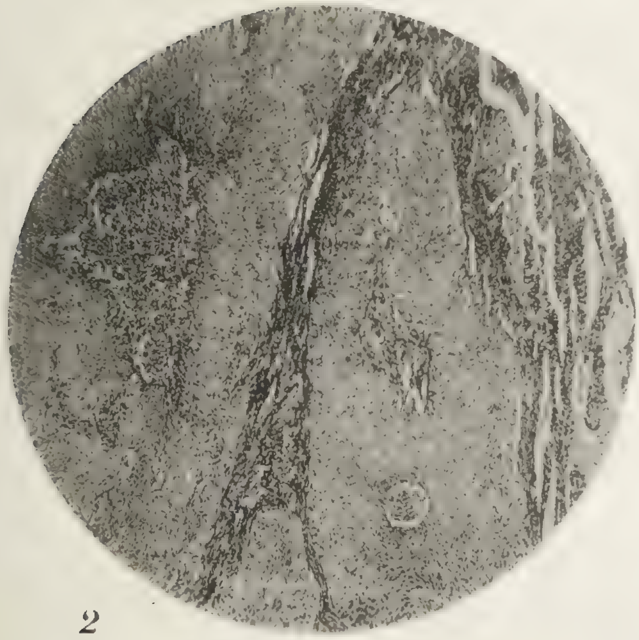

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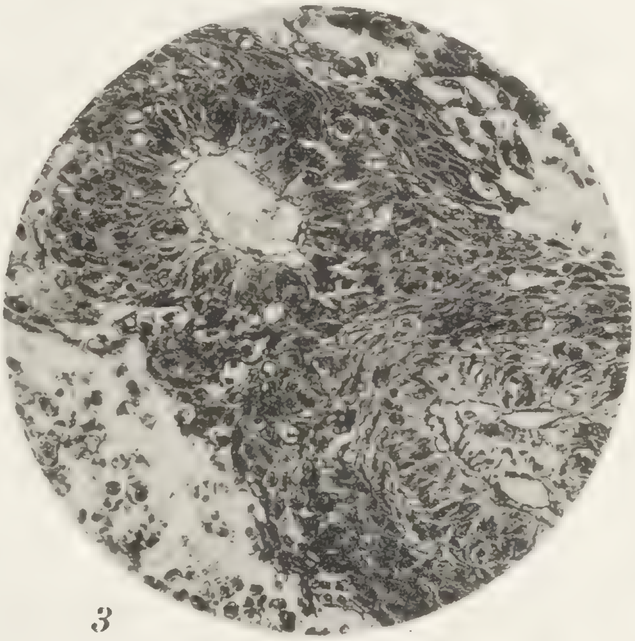

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Cancer of the Ovary in a Girl, Aged Eleven Years.

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CANCER OF THE OVARY IN A GIRL, AGED ELEVEN YEARS.

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The occurrence of cancer of the ovary at the age of eleven years, the misleading symptoms, the fact that a complete autopsy was done, and the material worked up thoroughly, makes this, the writers believe, a case well worth reporting.

The history of this patient is as follows: Miss B., aged eleven years, schoolgirl. The family history is unimportant.

Past History. Four months ago she was operated upon in another state for appendicitis. (No pathological report.) The appendix was removed and several small cysts in both ovaries punctured. From this operation the patient made an uneventful recovery, so far as healing of the wound was concerned, but she did not regain her strength. Soon she began to lose weight and ran an irregular temperature. She became pale and pasty in appearance, and spent most of her time in bed, complaining at times of pain in her lower abdomen over both sides.

Present Illness. Six weeks later one of us saw the patient on account of her right knee, which was swollen, somewhat hot, full of fluid, and quite tender—so much so that she could not walk upon it. No other parts being involved, a supporting bandage, ham splint, and rest in bed were advised, and under this treatment her family physician reported that the swelling soon reduced and the pain disappeared.

Six weeks later she was again seen on account of vomiting of several days' duration, increasing emaciation, and general enlargement of the abdomen. She was found much more emaciated, complaining of almost constant epigastric pain, vomiting nearly everything taken, and her abdomen was tensely distended by fluid. On palpation, it was possible to make out a hard irregular tumor in her epigastrium, about the size of a large grape fruit. On vaginal examination little could be gained, as the tense abdominal wall, small vagina and hymen prevented satisfactory palpation. The temperature had been irregularly elevated, but was never over 100.4° in the evening. The leukocytes were 10,800. The urinary findings were negative.

An operation was advised, as it seemed likely that the condition might be tuberculous peritonitis, possibly overlooked at the operation for appendicitis.

Operation. A median incision was made between the umbilicus and pubis, and a large amount of cloudy fluid let out of the abdomen. The hand was then inserted, and everywhere large nodular masses were found.

About the stomach there was a large mass running parallel with the transverse colon. Both ovaries were about equally enlarged, being two or three times their normal size. Their surfaces were symmetrically studded over with nodules, each about the size of a small English walnut. The ovaries themselves were hard, solid, and of abnormal consistency. The pelvis was filled on all sides by a hard nodular growth. Numerous nodules were felt along the root of the mesentery of the small intestine, and everywhere the bowel was bound down by adhesions to these masses. The cecum was examined for the appendiceal stump, but none could be found, as it had been inverted.

After this hasty examination it was at once evident that the condition was hopeless, and not to be improved by further operation. The right ovary was freed, its vessel ligated, and the organ removed for pathological examination. The abdomen was then hastily sutured, and the patient returned to bed.

Following the operation the abdomen again filled up with fluid, vomiting continued, and the patient gradually failed until the twenty-eighth day, when she died. An autopsy was performed by one of us (Dr. S. R. Haythorn), then of Harvard Medical School Pathological Department, and the report of the presence of an adenocarcinoma and the pathological findings, together with photomicrographs of the tumor, are submitted below.

This case was misleading in that four months before, the abdomen had been opened, its contents explored, and the appendix removed. At that time both ovaries were pulled into the wound to puncture cysts, so that their consistency and size were necessarily noted. The gradual decline, the accumulation of fluid, and the occurrence of an effusion in the knee-joint naturally inclined one toward the diagnosis of tuberculous peritonitis, possibly overlooked during the operation for appendicitis. The rarity of carcinoma at such an age, and the rapid development of all symptoms directly after operation, made the case almost, it seems to the writers, an impossible one to diagnosticate. Had there been less fluid in the abdomen, and had the vagina been larger and without a hymen, evidence might have been obtained from which correct conclusions might have been drawn.

It would seem from the autopsy findings, carcinoma not having been discovered elsewhere than in the ovaries and lymphatics, that there can be only two hypotheses as to its occurrence: (1) A secondary implantation of cells from a carcinomatous appendix directly into the ovaries; and (2) the primary development of carcinoma in the ovary itself, with metastasis into the opposite ovary.

In favor of the first theory is the occurrence of symptoms of appendicitis. Many of the cases of cancer of the appendix have been in young children, and have been operated upon with a diagnosis of appendicitis. One of the writers has observed such a case with typical symptoms of chronic appendicitis. Additional evidence in favor of secondary implantation is the equality in the size of both ovaries and the symmetrical arrangement of nodules in each; the preparation of the ovaries to receive implantations by puncturing of cysts; and the crushing of both tip and base of appendix in its removal, which is known to have been done.

In support of the second theory is the well-known fact that cancer is sometimes primary in the ovary, particularly in young girls, and that it frequently metastasizes into the opposite ovary. Here it must be said that at least some inequality in size between the primary and secondary growth would be expected; although this is not necessarily true, since elsewhere in the body it has frently been proved that metastases may outstrip primary growths. The most conclusive evidence in favor of the second explanation is the finding of tumor cells in the ovaries that exactly correspond to the cells found in the metastatic growths. Here we have no supposition, but a fact, and beyond this we can only conjecture.

In going over the literature, it is evident that carcinoma of the ovary in children is very uncommon, but not a rarity. Numerous cases are reported, some of which are carcinomatous degeneration in mixed tumors, others cancerous degeneration of cysts, and others primary carcinoma developing in the ovary itself. Many of the recorded cases are unaccompanied by pathological reports, or at best have only meagre ones, making it almost impossible to group them so that they are of any value. From the writings of others, many points which have been of interest to the writers, may also prove so to others who have not had occasion to go over the literature on this subject.

Many of the cases reported have been operated upon, as was this one, with a diagnosis of tuberculous peritonitis, principally on account of the presence of fluid in the abdomen without anasarca. This has been the most constant sign in the recorded cases, not more than 2 or 3 cases having been reported without it. Other signs which should make one suspicious of malignancy are: Pain, rapid emaciation, cachexia, cessation of menses, and edema of the extremities; particularly bilateral tumors; also hard and knotty ones of rapid growth, and proof of metastasis (Baader[1]). Another condition spoken of by reporters of ovarian tumors in children is the occurrence of early puberty, said sometimes to disappear after the tumor has been removed.

The diagnosis between carcinoma and sarcoma must only rarely be possible. Certain distinguishing signs are pointed out by Berent,[2] however. It is said that sarcoma is almost always primary, and if secondary, is, as a rule, from a uterine tumor, while cancer metastases in the ovaries, even from distant foci, such as stomach and breast, are not at all uncommon. Sarcomas are also said to metastasize and form adhesions later than carcinomas. In regard to size, sarcomas are apt to become much larger than carcinomas. It is also stated that carcinoma in relation to the ovary has a greater preference for young people than sarcoma. This we believe to be doubtful, in view of the number of cases of sarcoma of the ovary reported in young people. Pfannenstiel reports that in a large series the average age for the occurrence of sarcoma of the ovary was thirty-two years, with 40 per cent. in individuals under twenty-five years of age.

It may, therefore, be concluded, that:

1. The accurate diagnosis of malignant tumors of the ovary in young girls seems rarely possible.
2. Fluid in the abdomen in children without general anasarca, provided adhesive pericarditis and cirrhosis of the liver may be ruled out, should always be investigated by exploratory laparotomy.
3. Ovarian tumors, praticularly in young girls, should be removed with the least possible delay.
4. The occurrence of metastatic nodules in the fossa of Douglas, or in any other location where they may be definitely made out, should be accepted as evidence of extension too great to permit the case to be benefited by operation.

The pathological findings in the ovary that was removed at the operation, as well as a synopsis of the autopsy protocol of this case, are as follows:

SURGICAL SPECIMEN FROM OVARY (Harvard, 1909, No. 766). *Microscopic Description.* The sections were of a rapidly growing medullary tumor, the cells of which varied considerably in size, but were similar in structure. The average cell was twice the diameter of a polynuclear leukocyte. The next most common cell was again as large, and usually possessed from two to four nuclei. A third type of cell was a little larger than a large lymphocyte. The cells were round, oval, indented or elongated, depending upon the compactness of the surrounding growth. There was constantly a wide margin of clear protoplasm about the one or more nuclei, which were globular in outline and slightly vacuolated, giving them a cart-wheel appearance. The nuclei stained deeply and were usually multiple. Occasional monasters and diasters were present. The cells were held in cords or nests by a fine, fibrous reticulum, to which they did not appear to be attached. A general parallel arrangement of these columns of cells was preserved. The connective tissue stroma was gathered together into bands which ran either to a thin capsule or to a central connective mass. The bloodvessels were found only in the connective-tissue strands. Lymph spaces filled with free tumor cells were seen. There was practically no inflammatory reaction present. Diagnosis: Solidly growing carcinoma of ovary.[3]

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[1] Ein Fall von Carcinom des Ovarium im jugendlichen Alter, Tübingen, 1895.

[2] Ueber Maligne Ovarialgeschwülste bei Kindern, Berlin, 1901.

ANATOMICAL DIAGNOSIS FROM AUTOPSY PROTOCOL. Carcinoma (primary in ovaries). General metastasis of lymphatic distribution occurring in ovaries, uterus, pleura, peritoneum, esophagus, stomach, small intestine, colon, pancreas, gall-bladder, adrenals, urinary bladder, diaphragm, abdominal muscle, skin, mammary glands, and peribronchial, cervical, axillary inguinal, mesenteric, and retroperitoneal lymph nodes.

Other conditions present but not described below were: Atelectasis of lungs, ascites, hydrothorax (double), hydropericardium, edema of the left leg and two healed operation wounds on the abdomen. The absence of secondaries in the organs, namely, heart, lungs, spleen, liver, and kidneys, was striking when compared to the extensive distribution throughout all the lymphoid structures examined.

Synopsis of Autopsy Protocol. The body was greatly emaciated, development slight. Cervical, axillary, and inguinal nodes greatly enlarged, firm, and infiltrated. The left inguinal nodes were enlarged and adherent in a mass over the region of the inguinal ring, causing compression of the femoral vein and edema of the left leg. Nodular extensions upward from this mass infiltrated the whole of the left lower quadrant of the skin and abdomen. Both breasts were enlarged and nodular in contour. The umbilicus protruded slightly. Two healed operation scars were seen on the abdomen—one in the appendix region and the other, 10 cm. long, was 4 cm. to the right of the median line to which it ran parallel.

Pleural cavities: Both pleural cavities contained several hundred cubic centimeters of turbid yellow fluid. On the left there was so great an amount as to completely compress the lung. On the sternum, the upper surface of the diaphragm, and the parietal pleura were numerous spheroidal nodules, white in color and firm in consistency, which extended out into the pleural cavities. The visceral pleuræ were marked by white cord-like lines, having occasional small white bodies situated upon them. These bodies varied in size from small spots to nodules 1.5 cm. in diameter. Two large ones upon the left side were pedunculated.

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[3] We wish to express our thanks to Dr. F. B. Mallory and to Dr. Oskar Klotz for their opinions as to the nature of this tumor, and as to its probable primary site.

Left lung: The nodules were most numerous between the lobes. The lung was collapsed, atelectatic, firm in consistency, and dark reddish gray in color.

Right lung: The right lung was larger than the left and was air-containing throughout. The peribronchial lymph nodes were enlarged, several measured 3 cm. in diameter. They were milk white in color, and of moderately firm consistency.

Pericardium and heart showed no tumor.

Peritoneal cavity: A large, white, friable mass, 5 cm. in diameter, lying in the rectus muscle, was bisected by the incision. The breasts contained many similar masses. A large washbowl full of fluid was removed from the abdominal cavity. The parietal peritoneum was studded with innumerable small, white, solid nodules. There was a large chain of closely approximated, encapsulated nodules averaging about 3 cm. in diameter, extending across the omentum between the stomach and the transverse colon. The omentum was free from fat; it contained several small sacs filled with straw-colored fluid. The small intestines were held firmly in place by the mesentery, between the layers of which were great masses of nodules, many of them 5 and 7 cm. in diameter. The cecum, ascending and descending portions of the colon were densely adherent to the parietal walls. The pelvis was so filled with similar masses that relations could not be made out.

Spleen: The spleen was relatively large. A secondary growth, 2.5 cm. in diameter, was situated in the hilum. The capsule and the splenic pulp were otherwise negative.

Gastro-intestinal tract: The esophagus showed a great number of small nodular secondaries beneath the mucosa. The stomach wall was generally thickened and everywhere studded by small submucous nodular secondaries. The small intestine was firmly fixed throughout its entire length by the large secondaries in the mesentery. Its walls were thickly studded with discrete small spheroidal masses, which lay beneath the mucosa, which were not ulcerated, but produced bulgings in the lumen and beneath the serosa. The cecum was firmly embedded in the pelvic mass. The stump of the appendix could not be found. The condition of the colon resembled that in the small intestine. The rectum was inseparably bound in a mass of new growth in the pelvis.

Liver: The liver was negative and no secondaries were found. There were new growths of a nodular type about the gall-bladder, the bile ducts, and in the gall-bladder wall.

Pancreas: The pancreas was irregularly club-shaped, measuring 14 x 7 x 6 cm. The enlargement was due to new growths involving the lymph nodes about the head of the pancreas, and occurring in smaller masses throughout its length. The new growths were from 3 to 6 cm. in diameter, and were apparently separated from the true pancreatic tissue by a thin fibrous membrane.

Kidneys: The upper pole of the left kidney was embedded in the new growth about the adrenal and separated from it by a fibrous membrane. The growth did not invade either kidney. The ureters and pelves were greatly dilated on account of the pressure of the pelvic tumor constricting the lower ends of the ureters.

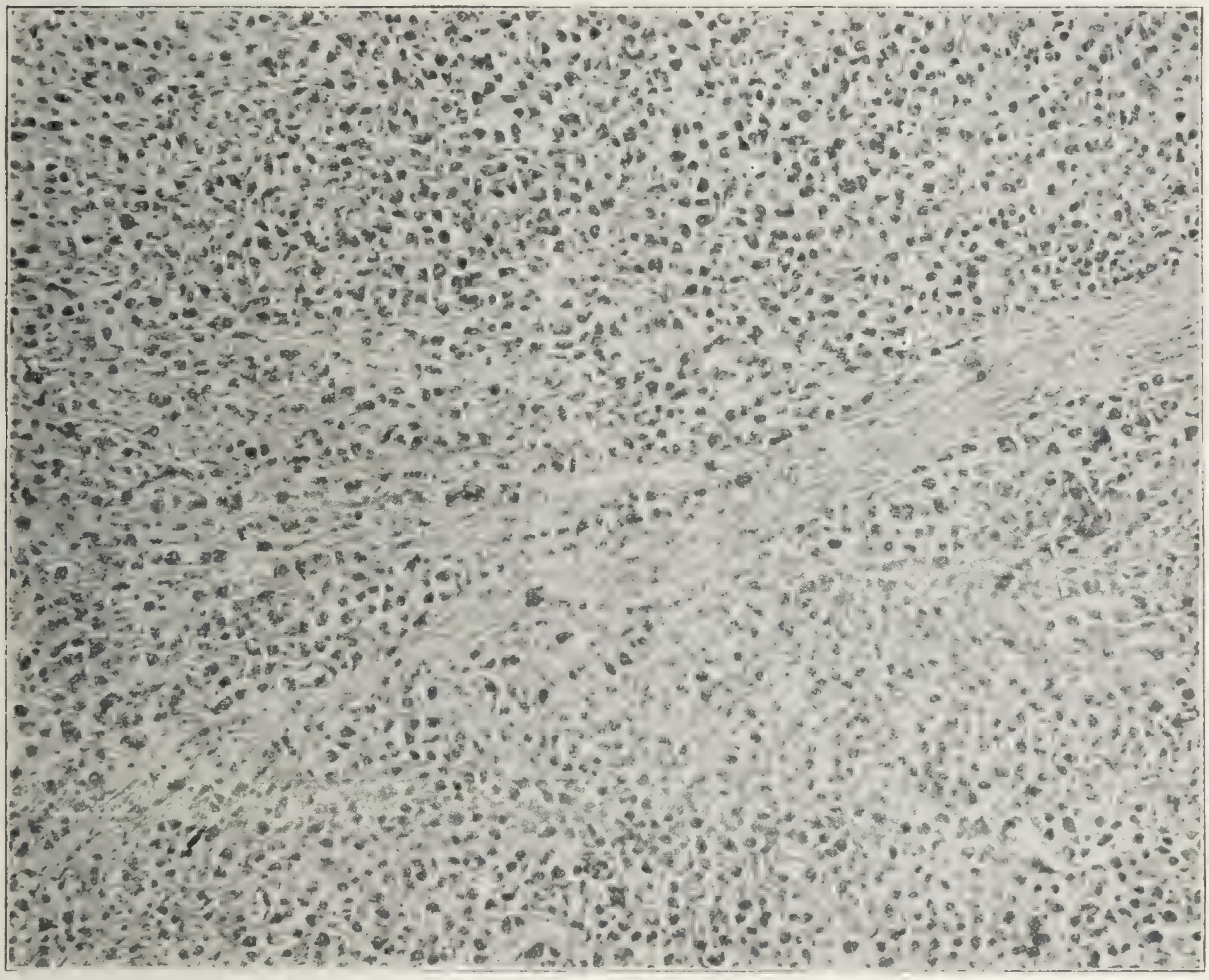

Fig. 1.—Section showing the typical structure of the tumor which was characteristic of the primary and all of the secondary growths. Objective Zeiss AA. Stained with hematoxylin and eosin.

Adrenals: About one-half of each adrenal was replaced by a globular new growth. These masses were much softer than those in other parts of the body. The one on the left measured 8 x 5 x 5 cm., and extended downward about the pole of the kidney and rested upon the upper surface of the renal vessels.

Aorta: The aorta was surrounded by masses of lymph nodes containing new growths. The lumen was negative.

Pelvic mass: The pelvis was filled with an adherent, infiltrating, friable tumor mass. A densely adherent, thick-walled cyst was found in the position previously held by the ovary removed at operation. It contained semifluid grayish-red material. The walls measured 2 cm. in thickness, were dark reddish-gray in color, smooth on the inner surface, and friable in consistence. The left ovary was 8 cm. in diameter, firm, hard, and adherent. The tubes and fundus of the uterus were all bound in an inseparable mass of reddish-gray tumor. This mass was continuous above with the retroperitoneal lymph nodes, and downward with a mass of nodes surrounding the femoral and inguinal rings and vessels.

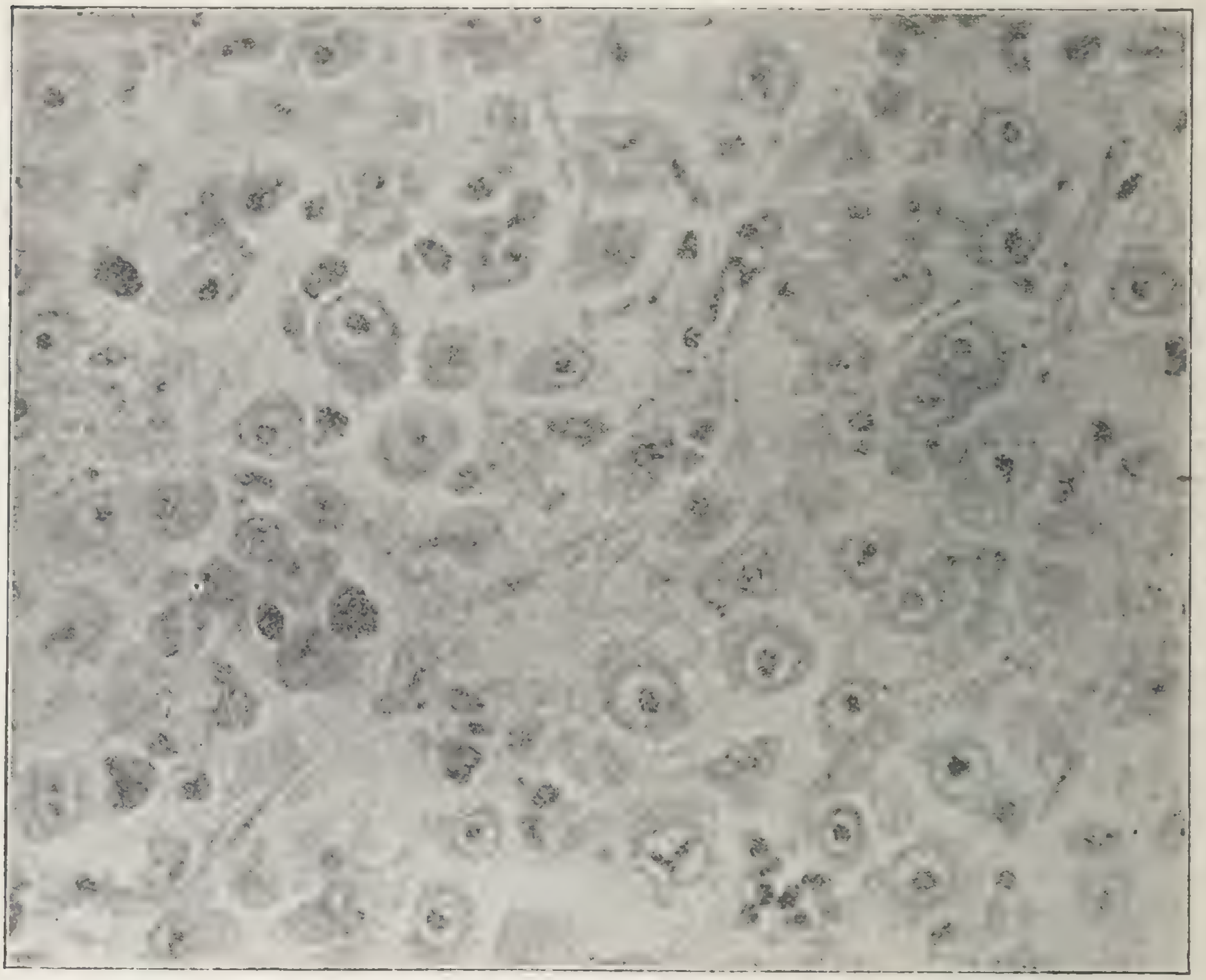

Fig. 2.—A cellular portion of one of the secondaries in the wall of the small intestine. Magnification Zeiss objective DD.

In taking up the microscopic findings of this case only those points are described which seem to have a more or less direct bearing on the tumor and the distribution of its secondaries. Other conditions which were found are simply mentioned. The microscopic appearances of all the sections which contain new growth are so constant, and so similar to those found in the ovary, removed surgically for diagnosis, that further description would cause unnecessary repetition (Fig. 1). The small nodules wherever found look like areas which might have been punched out of the tumor described above. Each one is provided with a very thin fibrous capsule. Hereafter they will be mentioned as secondaries.

Lung: In portions they were completely atelectatic. There was no tumor in the lung proper. Included within some of the lymph spaces of the pleura were typical secondary tumor nodules. In the neighboring lymph spaces and in the lymph spaces of the interlobular septa there were colonies of free tumor cells.

Spleen: The spleen was free from secondaries. The tumor mass at the hilum did not extend through the capsule.

Stomach: Portions of the stomach mucosa were practically normal. These passed gradually into the areas where the crypts had disappeared, and the mucosa had taken on the appearance of the tumor. The surfaces of these areas were necrotic, showed masses of bacteria, and were infiltrated with polymorphonuclear leukocytes. No true ulcers were found. The tumor of the mucosa extended downward to the greatly thickened muscularis mucosa. Numerous secondary tumor nodules were found in the submucosa. The subserous lymph spaces were dilated and filled with free tumor cells. The muscularis showed no secondaries.

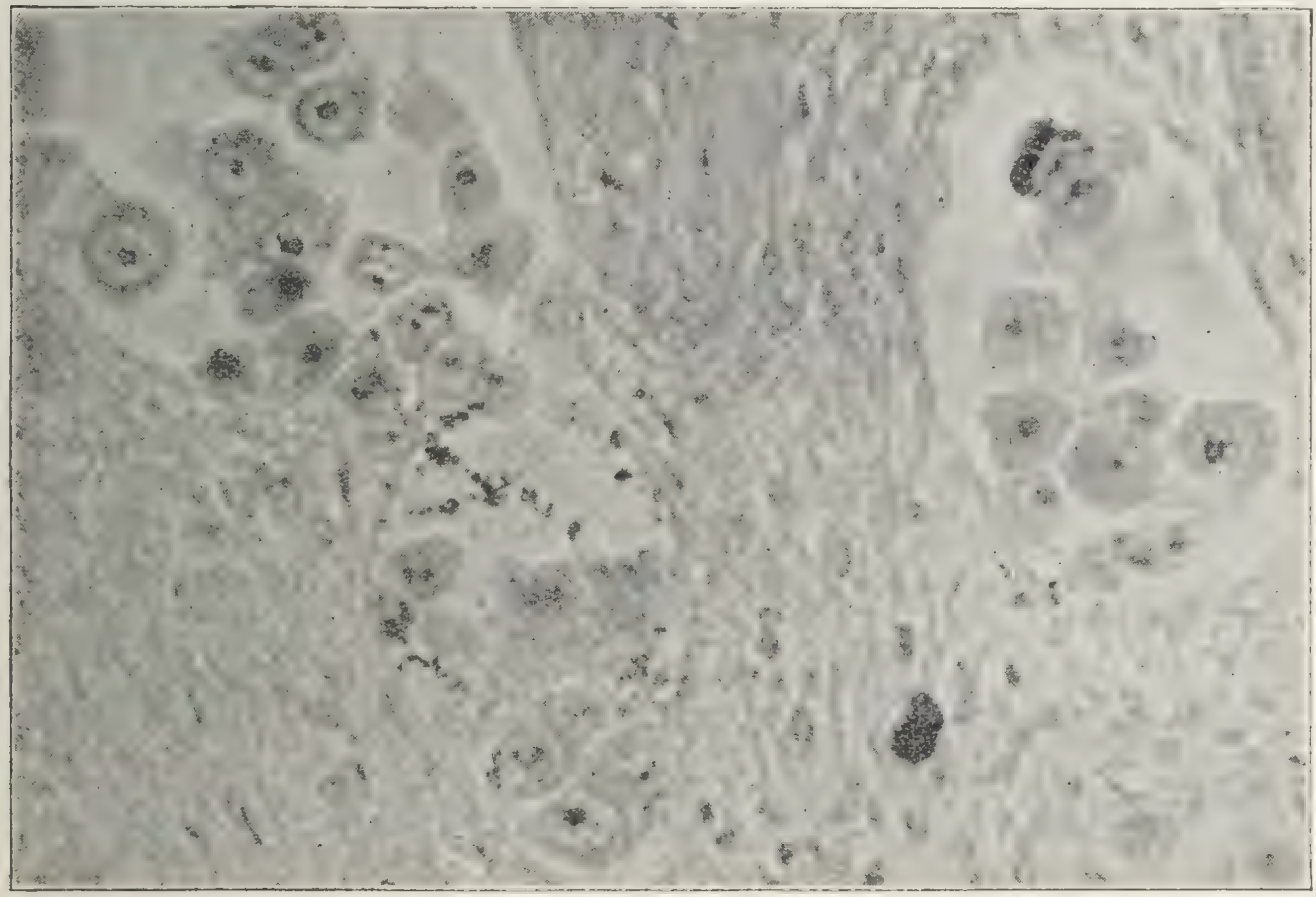

Fig. 3.—Lymph sinuses in the neighborhood of a secondary growth of the mesentery containing free tumor cells. Objective Zeiss DD.

Small intestine: Sections from the upper part of the small intestine showed that the lymphoid tissue had been replaced by tumor tissue, which sometimes appeared as polypoid growths on the sides of the valvulæ coniventes. The connective tissue of the valvulæ was infiltrated with masses of secondaries. The muscular layers were not affected and served to separate the small submucous nodules from the large tumor masses in the mesentery. Sections from the lower part of the small intestine showed the mucosa had been replaced by ordinary granulation tissue (Fig. 2). There were numerous discrete secondary nodules in the submucosa. The dilated lymph spaces of the mesentery were filled with free tumor cells. Within several veins there were seen, lying free among the red blood corpuscles, cells which to all appearances were tumor cells. These could easily be distinguished from endothelial cells which had been stripped from the vessel walls. Both types of cells were seen in some of the veins.

Pancreas: The pancreatic tissue appeared normal, and was separated from the tumor by fibrous bands. The secondary nodules in the pancreatic lymphatics showed areas of necrosis, in which the parallel arrangement of the stroma was especially well seen. Veins containing tumor cells were seen here in the borders of the secondaries.

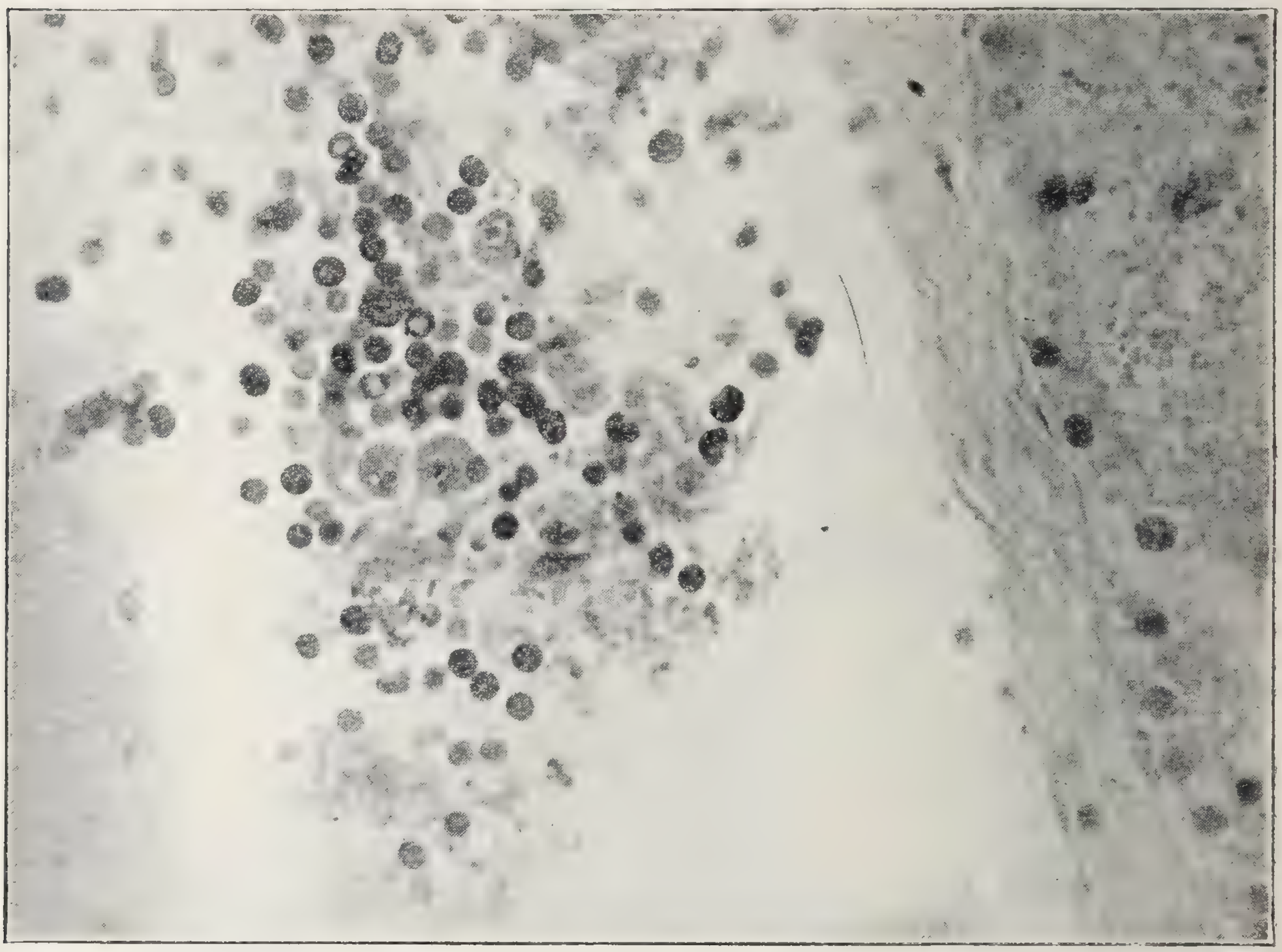

Fig. 4.—Small vein of the mesentery containing free tumor cells. Zeiss objective DD. The background immediately around the free tumor cells was slightly retouched.

Liver: No secondary growths were found. In the sinusoids and in some of the vessels there were rather numerous large cells. These cells resembled poorly stained tumor cells, were three to five times the diameter of a red corpuscle, had a distinct margin of protoplasm, a poorly stained nucleus, and one or more nucleoli, which were twice the size of the nuclei of the neighboring cells.

Kidneys: There were no secondaries. In some of the veins cells resembling tumor cells were seen. A mass of secondary growth was separated from the kidney by the fibrous capsule.

Adrenals: These showed large nodules of secondary growth.

Pelvic tumor: Sections from the pelvic mass showed the typical tumor growth. Here the connective tissue reticulum was more pronounced, the multinucleated cells relatively more numerous, and nuclear figures more common.

Summary. To sum up, we have a rapidly growing, malignant tumor, which produced very extensive metastases of lymphatic distribution. The structure was that of an epithelial growth of a round- or oval-celled type, which always reproduced the same picture and never developed cylindrical cells or became scirrhus. These points, added to the fact that the growth was first seen in the ovaries, leads to the supposition that it was primary in one or both of them. Most interesting were the cells in the mesenteric veins and in the liver, which appeared to be free tumor cells (Figs. 3 and 4). In the mesenteric veins there seemed to be little doubt that they were tumor cells, for they exactly resembled those in the lymph spaces, had nucleoli, and were larger than desquamated or wandering endothelial cells. In the liver they were not so well stained, but could be differentiated from displaced liver cells, in that they had larger nuclei, and were free from fat globules and pigment granules, while there was abundant pigment in the neighboring liver cells. Those found in the liver sinusoids were distinguished from wandering endothelial cells with difficulty, their greater size being the only obvious point of difference. None of these cells was found in the lung capillaries. The customary teaching on these tumors which metastasize wholly by the lymphatics is that the cells do not gain entrance to the blood stream; but granting that the cells observed were tumor cells, another explanation must be sought in this case. Three possibilities have suggested themselves: (1) That the blood had closely associated with it (maybe with the red cells) an immune body which brought about the destruction of the tumor cells, but did not diffuse through to the lymph. (2) That the tumor cells were carried to the liver by the portal circulation and destroyed. (3) The most likely possibility of all is that the question was a quantitative one, the lymphatics in the neighborhood of new growth being filled with great numbers of tumor cells, while some search was required to find those in the veins.

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LIFE OF PASTEUR

by

C. H. Marcy, A. B.
(Pathological Laboratory, University of Pittsburgh).

We can appreciate more fully the work of Pasteur if we look back on the condition of medicine as a science at the time he started his studies and see how little was really known about the actual cause of disease. The Greek physicians gave excellent accounts of many diseases; they knew the mode of termination and had studied the art of prognosis carefully, but of the actually cause they knew little or nothing. Their treatment was haphazard, partly the outcome of experience and partly based on false theories. It was not until man began to study the "seats and causes" and to search out the changes inside the body corresponding to the outward symptoms that anything definite was known. Morbid anatomy

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began to be studied in about 1750 and in the hundred years following, such advances were made that the post-mortem appearance of the more common disease was known. Great advances, too, were made in treatment but very little was based on scientific knowledge, in fact at the middle of the last century not much more was known of the actual causes of the great scourges of the race, the plagues, the fevers and the pestilences than did the Greeks. It was known that fevers were contagious, that diseases spread and became epidemic, but the reason was a mystery. Many theories were offered in explanation probably foremost of which stood "spontaneous generation." It was in the darkness Pasteur geban his work.

Pasteur's ancestirs can be traced back as far as 1682. His father, Joseph Pasteur, was a tanner by trade, had served in the army under Napoleon and had been made a member of the Legion of Honor for distinbegan to be studied in about 1750 and in the hundred years following, such advances were made that the post-mortem appearances of the more common diseases was known. Great advances, too, were made in treatment but very little was based on scientific knowledge, in fact at the middle of the last century not much more was known of the actual causes of the great scourges of the race, the plagues, the fevers and the pestilences than did the Greeks. It was known that fevers were contagious, that diseases spread and became epidemic, but the reason was a mystery. Many theories were offered in explanation probably foremost of which stood "spontaneous generation." It was in the dilemma Pasteur began his work.

Pasteur's ancestors can be traced back as far as 1683. His father, Joseph Pasteur, was a tanner by trade, had served in the army under Napoleon and had been made a member of the Legion of Honour for distintinguished service. His mother, Jeanne Pasteur, belonged to one of the oldest plebian families in France. This couple lived a quiet life at Dole where Louis Pasteur was born Friday, Dec. 27th, 1822. Soon after his birth his parents moved to Arbois. It was here Louis began his education, entering the primary department of the College of Arbois. Records show him to have been a good average student with apparent desire for education and no particular talent for anything but drawing. About the age of fifteen he became acquainted with an old army physician, Dr. Beaumont, whose tales of medical work are supposed to have had a great influence on young Pasteur's life. The head master of the College, taking a special interest in him, opened up the possibilities of a higher education at the "Ecole Normale" in Paris, so in 1838 at the age of 16 he set out to study for his pre-requisite "Baccalauret" degree.

However, becoming homesick in Paris his father recalled him at the end of the first year and again sent him to Arbois College to continue his study of drawing. Not satisfied with this, and still with the desire to enter the "Ecole Normale," he decided to take his "Baccalauret" at the Royal College at Besoncon which was nearer home. In August 1842 being declared admissable to the examinations, but standing only fifteenth out of twenty-two candidates, he thought it best to spend one year more in Paris in preparation before entering. During his early work at Paris he became very much interested in the lectures of J. B. Dumas on chemistry and concentrated his energies along this line. In the chemical laboratory his attention was attracted to tartaric and paratartaric or racemia acid then being studied by Mitscherlich and Biot, who had found that tartrate and paratartrate of soda and ammonia had similar crystaline form, the nature and number

of atoms, their arrangement and distances were the same, yet dissolved tartrate rotated the plane of polarized light, and the paratartrate remained inactive; the explanation of this was not known. Pasteur working on this in his spare moments, noticed that the crystals of tartaric acid and the tartrate had little faces which had escaped even the profound observations of Mitcherlich and Biot. The faces which existed only on one-half of the edges or similar angles constituted what is called a hemihedral form. On examining the crystals of paratartaric acid he found them also hemihedral but the faces of some were inclined to the right and those of others to the left, sorting these carefully, putting on one side those which turned to the left, and on the other those which turned to the right, these piles when dissolved made solutions which rotated the plane of light to the left and right respectively, and on being mixed together, as in of atoms, their arrangement and distances were the same, yet dissolved tartrate rotated the plane of polarized light, and the paratartrate remained inactive; the explanation of this was not known. Pasteur working on this in his spare moments, found that the crystals of tartaric acid and the tartrate had little faces which had escaped even the profound observations of Mitscherlich and Biot. The faces which existed only on one-half of the edges or similar angles constituted what is called a hemihedral form. On examining the crystals of paratartrate he found them also hemihedral but the faces of some were inclined to the right and those of others to the left, sorting these carefully, putting on one side those which turned to the left and on the other those which turned to the right, these gave when dissolved each solutions which rotated the plane of light to the left and right respectively, and on being mixed together, as in the original state, exactly neutralized each other, thus explaining the inactivity of paratartrate. By this discovery Pasteur had opened up an immense field in Chemistry hitherto unknown. Shortly after this he was sent to Strassburgh to teach and while there in May, 1849 married the daughter of the Rector of Strassburg Academy. By this time his work on racemic acid and his studies on crystalography had been recognized and approved by the leading scientists of the country but not satisfied he set out to manufacture racemic acid, which up to this time had only been observed in small quantities. Visiting chemical works all over the continent where traces had been seen, and after many experiments in the laboratory following these observations, he succeeded in producing tartaric acid artificially, by maintaining tartrate of cinchonin at a high temperature for several hours; and from this was able to isolate definitely, destro-tartaric, levo-tar-

taric, racemic and meso-tartatic acid. In recognition of this work he was awarded a prize of fifteen hundred francos and made a member of the Legion of Honor. Continuing this work he observed that one of the salts of racemix acid, racemate or ammonia, when placed in ordinary conditions of fermentation, the dextro-tartatic acid alone fermented. These interesting experiments which were turning his attention toward fermentation were broken by a call to Lille as dean of the faculty of science. Here again we see the systematic methods Pasteur used in all his work showing up in his teaching. Great care was taken in the preparation of all lectures, systems were installed by which students could enter and practice experiments in the laboratories; interest was maintained in the studies by introducing of inspection to the different industries in the city.

In the summer of 1856 a Lille mantartic, racemic and meso-tartaric acid. In recognition of this work he was awarded a prize of fifteen hundred francs and made a member of the Legion of Honor. Continuing this work he observed that one of the salts of racemic acid, racemate of ammonia, when placed in ordinary conditions of fermentation, the dextro-tartaric acid alone fermented. These interesting experiments which were turning his attention toward fermentation were broken by a call to Lille as dean of the faculty of sciences. Here again we see the systematic methods Pasteur used in all his work showing up in his teaching. Great care was taken in the preparation of all lectures, systems were installed by which students could enter and practice experiments in the laboratories; interest was maintained in the studies by introducing of inspection to the different industries in the city.

In the summer of 1856 a little manufacture met with great disappointment in the manufacture of beet root alcohol and came to Pasteur for advice. The phenomena of fermentation which had preoccupied him to such a degree while in Paris caused him to make some experiments. Time was spent daily at the factory observing conditions, on return to his laboratory he studied the globules in the fermentation and compared filtered with non-filtered juices. The small globules were observed to be found when fermentation was healthy, lengthened when alteration began and quite long when fermentation became lactic; by this method it was possible to follow the changes taking place. He was on the road to a disæovery the consequences of which were to revolutionize chemistry. For months and months he worked to assure himself he was not the prey to error. Up to these obserfations practically nothing of the act of fermentation was known. All that two of the greatest author-makers met with great disappointment in the manufacture of beet root alcohol and came to Pasteur for advice. The phenomena of fermentation which had preoccupied him to such a degree while at Lille caused him to make more experiments. Time was spent daily at one factory observing conditions, on return to his laboratory he studied the globules in the fermentation and compared filtered with non-filtered juices. The small globules were observed to be round when fermentation was healthy, lengthened when alteration began and quite long when fermentation became lactic; by this method it was possible to follow the changes taking place. He was on the road to a discovery the consequences of which were to revolutionize chemistry. For months and months he worked to educate himself as [illegible] [illegible]. Up to these observations practically nothing of the art of fermentation was known. All that was

of the previous generities of the time, Dumas and Bernard, could say about it was that it was strange and obscure. Day after day he drew in his note book the little globules, the tiny bodies he found in a grey substance sometimes arranged in a zone. These globules had escaped the observations of chemists and naturalists because of the ease to confound them with the many other products of lactic fermentation. After isolating and scattering in a liquid a trace of this grey substance Pasteur saw some characterized lactic fermentation appear, the grey substance was indeen the ferment. This work was leading him into seemingly impenetrable mystery, the celebrated question of "spontaneous generation." The advice of Dumas and Biot not to enter this field as he would never find his way out stimulated his attempt to fathom this unsolved proplem. Spontaneous generation was believed in at this time by most ities of the time. Dumas and [illegible], could say about it was that it was strange and obscure. Day after day he drew in his note book the little globules, the tiny bodies he found in a gray substance sometimes arranged in a chain. These globules had escaped the observations of chemists and naturalists because at the came to confound them with the many other products of lactic fermentation. After isolating and scattering in a fluid a trace of this gray substance without any with sugar started lactic fermentation again, the gray substance was indeed the ferment. This work was leading him into seemingly impenetrable mystery, the oft-debated question of "spontaneous generation." The advice of Dumas and Biot not to enter this field as he could never find his way out stimulated his attempt to fathom this unsolved problem. Spontaneous generation was believed in at this time by many scientists of the world. Pasteur took for his starting point the microscopic study of the atmospheric air, basing his labors on the supposition that if germs existed in the air they could be filtered out. After much work he found that by drawing air through cotton wool and then placing this wool in a pure liquid certain alterations were set up in the liquid. Next flasks were prepared with long curved necks, there were filled with an alterable liquid or media and the contents boiled, when set set aside no alterations occurred, but if the liquid were run in the curved part of the tube where dust had collected soon alterations took place. Again he took flasks containing boiled media and sealed the necks. These were then opened allowing air from different rooms to enter, some of the media was altered, while others was not, depending on the locality in which opened, the experiment was repeated by taking twenty scientists of the world. Pasteur took for his starting point the microscopic study of the atmospheric air, basing his theory on the supposition that if germs existed in the air they could be filtered out. After much work he found that by forcing air through cotton wool and then placing this wool in a pure liquid certain fermentations were set up in the liquid. Next flasks were prepared with long curved necks, these were filled with an albuminous liquid or media and the contents boiled, they not only showed no fermentation produced, but if the liquid were put in the curved part of the neck where dust had collected, fermentations took place. Again he took flasks containing sterile media and sealed the necks. These were then opened at very different places in Paris, some of the media was affected, while others were not, depending upon the locality in which opened. The experiments thus succeeded in proving beyond sealed flasks and ascending the Alps several thousand feetn opening the flasks for a short time and again sealing them; he found that out of twenty exposed only one became altered. Satisfying himself that in the air existed certain germs producing certain alterations in media, he again turned to the subject of fermentation, endeavoring to determine the nature of the ferment. During his study he found butyric acid fermentation due to small cylindrical rods with rounded ends, and that when sown upon suitable media grew media without requiring the least particle of air, in fact air destroyed the growth and CO_2 did not affect it, to these he gave the name anaerobes. Applying these observations to wines he decided that the so-called diseases of wine were due to organized ferments, when certain circumstances of temperature, and exposure to air favored their evolution or introduction into wines. Here also he found that by heating a wine at a sealed flasks and ascending the Alps several thousand feet, opening the flasks for a short time and again sealing them; he found that out of twenty exposed only one became altered, satisfying himself that in the air existed certain germs producing certain alterations in milk, he again turned to the subject of fermentation, endeavoring to determine the nature of the ferment. During his study he found butyric acid fermentation due to small cylindrical rods with rounded ends, and that when sown upon suitable media grew media without requiring the least particle of air, in fact air destroyed the growth and Oxyg did not affect it, to these he gave the name anaerobes. Applying these observations to wines he decided that the so-called diseases of wine were due to organized ferments, when certain circumstances of temperature, and exposure to air favored their evolution or introduction into wines. Here also he found that by heating a wine at a certain desirable point in its process of fermentation to a temperature of 50 to 60 degrees C. would destroy all vitality of the germs and still not affect the improvement of ageing which he said was due not to fermentation, but to a slow oxidation favored by heat. This"Pasteurization" of wines as it was called was of immense value to manufactures, as thousands of dollars were being lost annually by souring, rendering the products unusable. At this time a great silk worm epidemic was causing millions of dollars damage to France. J. B. Dumas Pasteur's old teacher, had been called by the country to find if possible some means of checking the ravages of the disease. Dumas thinking Pasteur more capable of the work, asked him to make an investigation. Pasteur not having any definite knowledge of the industry started to study the subject of silk worms in order to have a clear foundation on which to base his excertain definite point in the process of fermentation to a temperature of 50 to 60 degrees C. would destroy all vitality of the germs and still not affect the improvement of ageing which he said was due to oxygenation, but this slow oxidation favored by heat. This "pasteurization" of wines as it was called was of immense value to manufacturers, as thousands of dollars were being lost annually by souring, rendering the product unsalable. At this time a great silk worm epidemic was causing millions of dollars damage in France. J. B. Dumas Pasteur's old teacher, had been asked by the country to find if possible some means of checking the ravages of the disease. Dumas entreated Pasteur to undertake the work which he did as an investigation. Pasteur not having any definite knowledge of the industry started to study the subject of silk worms in order to have a clear conception on which to base his experiments. Establishing a temporary laboratory at Allais in the heart of the silk worm country, he began a systematic study of the various stages of their development. He first noticed that the diseased worms or moths contained peculiar corpuscles and that healthy moths coming in contact with these also became diseased. In order to stop the disease it immediately appeared necessary to keep the corpuscled and non-corpuscled moths separate, however another difficulty confronted him, out of a brood of a hundred worms he was raising with great care fifteen to twenty were dying daily showing no trace of corpuscles. The difficulty was only temporary, however, for in the intestinal canal of these dead worms he discovered organisms which produced a well characterized example of fla~~t~~chery disease, a disease independent and distinct from pebrine. With this knoweldge he was able to prove

that every moth showing corpuscles or containing the flatchery vibris must give birth to diseased seed which in turn would produce diseased worms. Suspected seed was now discarded and only that known to come from healthy moths used. The healthy colonies raised by this new Pasteur system were kept away from infected areas and it was not long until the industry had reached the high rank it once held. In 1870 his research work was suspended because of the Prussian invasion of France. Pasteur's son, then 18 years old, enlisted in the army, Pasteur himself wished to enter active service, but his friends compeeled him to retire to Arbois on account of his ill health, having suffered fro a slight stroke of paralysis during his strenuous work on the silk worm disease.

Resuming his work at the close of the war he took up the study of the manufacture of beer. Great trouble was being had in the beer becoming acid, sour, sligy or

putrid. Pasteur thought this porbably due to the development of germs in the air, in the water or on the surface of the numerous utensils used in the brewery. He reported his previous tests on fermentation, using beer as a medium, allowing it to stand in the air, etc. On a visit to the large breweries in England he found in all cases of altered beer the micro-organisms foreign to the true beer yeast. From the various cafes he purchased samples and after letting them stand for twenty-four hours decanted them and sowed on drop of the sediment in each vessel, kept these at a temporature of twenty degrees C. for fifteen to eighteen days and found at the end of that time all contained ferments of disease. He then sowed pure yeast in other vessels with the same precautions and all the beer of this series remained pure from strange ferments and free from bad taste. Concluding he laid down the three following

principles:-

(1) Every alteration of the wort or of the beer itself depends on the development of micro-organisms, which are the ferments of disease.

(2) These germs of ferment are brought by the air, by the ingredients, or by the apparatus used in the breweries.

(3) Whenever beer contained no living organisms it is unalterable.

These principles, formulated and proved, were to triumph over all professional uncertainties, and in the same way wines could be preserved and beer could be successfully treated. Thus a new word "Pasteurized" beer became current in technical language.

Pasteur with his work on ferments had gotten glimses of another world, the disease virus ferments. After the service rendered during the last ten years to vine-

gar makers, silk worm cultivators, vine growers and brewers, he now wished to tackel what had been on his mind since 1861, the study of contageous diseases. His own sorrows had only made him incline more toward the grief of others. The memory of the children he had lost, the mournings he had witnessed, caused him to passionately desire that there might be fewer empty places in desolate homes. He regretted that he was not a medical man, as he saw that this would aid him in knowing and describing exactly the course of disease. An unexpected offer assisted in filling this, what he considered a blank. At the beginning of the year 1873 he was elected to the Academy of Medicine, immediately promising himself that he would be a most punctual attendant. As he walked toward the desk alloted him at the first meeting little did his colleagues suspect [illegible] industry, with more cultivation, wise success and strength, he was raised to [illegible] for years to his mind alone [illegible]. The study of engineering throughout. All its questions had only made him feeling more toward the growth of [illegible]. The suc- cess of the [illegible] he had [illegible], the [illegible] he had witnessed, convinced him to posi- tively decide that which stood in his way an early aiming at something higher. He re- alized that he was not a medical man, and he saw that this would aid him in becoming [illegible] the change of his course. He wrote and after [illegible] in filling this, that he considered a change. At the beginning of the year 1877 he was admitted to the faculty of Medicine, im- mediately receiving himself that he would be a most practical physician. As he relates about his days, [illegible] him at his first meeting little [illegible] his colleagues respect that this quiet and unassuming new member would become the greatest revolutionary ever known to medicine. At this time the medical profession had no use for experimental laboratory methods, they called Claude Bernard's work a science de lux which could well be dispended with. One surgeon in a speech before the Academy stated that "laboratory surgery" had destroyed many animals and saved very few human lives. Pasteur in repeated speeches and arguments based on his experiments tried to convince them that it was germs that were causing the infections which were producing so much death. The mortality for amputations alone was sixty per cent. People was dying in the hospitals by the thousands of gangrene and septicaemia, a pin prick was the open door to death, wounds of the soldiers in the hospitals were filled with pus. He

demonstrated how it would be possible

that this quiet and unassuming new worker would become the greatest revolutionary ever known in medicine. At this time the medical profession had no use for experimental laboratory methods, they called Claude Bernard's work a science de lux which could well be dispensed with. One surgeon in a speech before the Academy stated that "laboratory surgery" and destroyed many animals and saved very few human lives. Pasteur in repeated speeches and arguments based on his experiments tried to convince them that it was germs that were causing the infections which were producing so much death. The mortality for amputations alone was sixty per cent. Sepsis was rife in the hospitals by the carelessness of surgeons and attendants, a pin prick was the open door to death. Wounds of the soldiers in the hospitals were filled with pus, the for virus ferments to be carried by dust in the air, on instruments, dirty dressings, etc., the same as the foreign ferments in beer. He advocated that all instruments used be passed through flame to destroy minute bodies which might collect in unobserved crevices; that the wounds should be cleansed with some solution like carbolic acid, which was supposed to destroy the living virulent organisms;that cotton-wool dressings previously heated should be used over the wounds to protect them from dust, andthat pure air should be maintained in the sick room. Medical men laughed at this theory and these precautions of a chemist, as he was called, until in 1867 Lister, a noted surgeon of England, reading Pasteru's work, decided to apply these methods to surgery. The air in the operating room was filled with carbolic spray,hands and instruments were cleansed with carbolic

acid, great care was taken with the dressings; the astounding result was that out of forty amputations thirty-four were successful. This was a start, it was not long until Pasteur's hygenic methods were adopted by other men in hospitals, with the result that the mortality from infections dropped enormously.

Progressing rapidly on his fight on infection, it was his great desire to isolate and combat successfully some definite infectous disease. Charbon or splenic fever was destroying thousands of sheep and cattle, as well as hundreds of men annually. Davaine in 1850 noticed little filiform bodies in the blood of animals dying from anthrax, but attached no importance to the fact. Eleven years later when reading Pasteur's paper on butyric action ferment he wondered if the filiform bodies said, great care was taken with the ligatures; the astounding result was that out of forty amputations thirty-four were successful. This was a start; it was not long until Pasteur's hygenic methods were adopted by other men in hospitals, with the result that the mortality from infections dropped enormously.

Interesting reports on his fight on infection. It was his great desire to isolate and combat successfully some infectious diseases. Anthrax or splenic fever was destroying thousands of sheep and cattle, as well as hundreds of men annually. Davaine in 1850 noticed little filiform bodies in the blood of animals dying from anthrax, but [illegible] no importance to the fact. Thirteen years later upon reading Pasteur's paper on butyric [illegible] thought of the filiform bodies found in the blood might not act after the manner of ferments and cause disease. He inoculated some rabbits with the blood, they died and the rod shaped forms were found in their blood. It might be thought the cause of the disease was found, but two other scientists refuted the experiments. They inoculated some rabbits with the blood of a cow, which had died of anthrax several days before, and found that while the rabbits died they did not present any bacteriaemia. It was therefore claimed that the bacteria was not the cause of the disease. Dr. Koch working at this time was able to grow the bacilli on artificial media and had observed the formation of spores. Pasteur taking up the study repeated Koch's experiments, and assuring himself of their truth he procurred a sheep that had died sixteen hours before, a horse that had died the preceding day, and a cow that had been dead three or four days with anthrax. Examining the blood of the found in the blood might not act after the manner of ferments and cause disease. He inoculated some rabbits with the blood, they died and the rod shaped forms were found in their blood. It might be thought the cause of the disease was found, but two other scientists refuted the experiments. They inoculated some rabbits with the blood of a cow which had died of anthrax several days before, and found that while the rabbits died they did not produce any bacteridia. It was therefore claimed that the bacteria was not the cause of the disease. R. Koch working at this time was able to grow the bacilli on artificial media and had observed the formation of spores. Pasteur taking up the study repeated Koch's experiments and assuring himself of their truth he experimented on a sheep that had died sixteen hours before, a horse that had died the preceding day, and a cow that had been dead three or four days with anthrax. Examining the blood of the sheep he found anthrax only, in the blood of the horse anthrax and putrefaction vibrinos, and in the cow anthrax and a still greater number of vibrinos. Inoculating other animals with this blood, those inoculated with the blood of the horse and cow died showing no bacteriaemia, but those inoculated from the blood of the sheep died showing presence of the bacillus anthracis. He then took a horse that was in perfect health, asphyziated it and drew blood from the deep veins, this he found contained septic vibrio which then inoculated into rabbits killed them in twenty-four to thirty-six hours. Successive cultures of the vibrio enabled him to show as he had done with anthrax, that one drop of those cultures caused spoticaemia in an animal, but while the anthrax grew arobically the vibrio grew anaerobically. Next he inoculated the sheep he found anthrax only in the blood of the horse anthrax and putrefaction vibriones, and in the cow anthrax and a still greater number of vibriones. Inoculating other animals with this blood, those inoculated with the blood of the horse and cow died showing no bacteridia, but those inoculated from the blood of the sheep died showing presence of the bacillus anthracis. He then took a horse that was in perfect health, asphyxiated it and drew blood from the deep veins, this he found contained septic vibrio which then inoculated into rabbits killed them in twenty-four to thirty-six hours. Successive cultures of the vibrio enabled him to show as he had done with anthrax, that one drop of those cultures caused septicaemia in an animal, but while the anthrax grew aerobically the vibrio grew anaerobically. Next he inoculated the vibrio from one culture tube to another until fifty had been inoculated to prove that the last would produce death as well as the first, so nn possible doubt could remain that this was the death producing factor. The next experiment consisted in cutting a sheep, introducing a drop of the septic vibrio culture, noting the gangrene produced, then using the infected instruments on another animal, and producing the same conditions; in this way he was anabled to study the laws of sterilization and prove definitely his hygenic theory advanced a short time before. The department of agriculture following this work asked him to investigate the cause of the splenic fever epidemic. Entering the country where the disease was most prevalent he watched the flocks of sheep in the pastures and observed that a sheep would suddenly become ill and die without

apparent cause, the dead sheep being later buried by the shepard. Wondering if the infection was not produced by some food he inoculated different food stuffs with spores and found that those foods producing abrasions in the mouth immediately infected the animal. Led by this to beleive that the source lay in the pastures, on a close examination it was found that where the dead animals were buried the earth covering them contained bacteria which could be cultivated on artificial media. The conclusion was obvious, worms working on the dead animals on coming to the surface carried the very resistant spores with them and these were picked up by the sheep in grazing.

While making these observations a head of a chicken that had died of chicken cholera, another wide spread epidemic, was sent to him for examination. In it

he found small micro-organisms which could be isolated and which in turn would produce the disease in other chickens. Inoculations were repeated at twenty-four hour intervals from tube to tube and the virulence remained the same. By chance one day when making some inoculations, he picked up an old culture, to his surprise the chicken inoculated became only slightly ill, and did not die; he inoculated it again with a newer strain and again to his surprise it produced no effect on the hen. Now by making inoculations from culture to culture at different intervals he produced attenuated strains which in turn would produce non-virulent organisms, which when injected into other chickens set up an immunity to virulent cultures. At last he had found the long sought for means of prevention of an infectious disease. Immediately returning

to his anthrax he was again able to produce an attenuated virus which made cattle and sheep immune from the deadly splenic fever. His highest ambitions had been accomplished; he saw before him unlimited fields. On Dec. 8th, 1882, he was elected to the French Academy, one of the greatest honors in the country, the University of Edinburg conferred their highest degrees upon him and the entire world recognized his wonderful work.

Amidst the various researches undertaken in his laboratory, one study was placed by Pasteur above every other, one mystery constantly haunted his mind, that of hydrophobia. Much confusion prevailed at this time regarding this disease, its seat, its cause and remedy. Three things seemed possible, that the rabic virus was contained in the salvia

of the mad animals, secondly, that it was communicated through bites; and thirdly, that the period of incubation might vary from a few days to seferal months. As the number of cases he observed became larger, he felt a growing conviction that hydrophobia had its seat in the nervous system, and particularly in the medulla oblongata. Post-mortem revealed no lesions, but when parts of the medulla were removed, ground up in sterile water and inoculated under the dog'd skin hydrophobia always resulted. It then occurred to him to inoculate the rabid virus directly on the surface of the dog's brain, this done characteristic hydrophobia appeared after fourteen days. Continuing these inoculations from the brain of one dog to that of another until a hundred uninterrupted inoculations had been made, the time of incubation became fixed at seven days. Pastuer nor abstracted of man and animals, secondly, that it was communicated through blood; and thirdly, that the period of incubation might vary from a few days to several months. As the number of cases he observed became larger, he felt a growing conviction that hydrophobia had its seat in the nervous system, and particularly in the medulla oblongata. Experiments revealed no lesions, but when parts of the medulla were removed, ground up in sterile water and inoculated under the dog's skin hydrophobia always resulted. It then occurred to him to inoculate the brain virus directly on the surface of the dog's brain, when the characteristic hydrophobia appeared after fourteen days. Continuing these inoculations from the brain of one dog to that of another until a hundred uninterrupted inoculations had been made, the time of incubation became fixed at seven days. Pasteur now abstracted a fragment of the medulla of a rabbit which had just died of rabies, after an inoculation of this fixed virus; this fragment was suspended by a thread in a sterilized phial; as the medulla gradually became dry its virulence increased, until the end of fourteen days it had become absolutely extinguished. This now inactive medulla was crushed and injected under the skin of some dogs, the following day they were inoculated with a medulla that had been drying thirteen days, and so on, using that of increased virulence until the medulla used was of a rabbit dead the same day. The dogs might now be bitten by rabid dogs or submitted to the intracranial imoculations of the deadly virus; they resisted both. On Monday, July 6th, 1885, a little boy accompanied by his mother entered the laboratory, the boy had been seriously bitten by a mad dog several days before. Pasteur was convinced that the boy's a fragment of the medulla of a rabbit which had just died of rabies, after an inoculation of this fixed virus; this fragment was suspended by a thread in a sterilized phial; as the medulla gradually became dry the virulence decreased, until at the end of fourteen days it had become absolutely extinguished. This now inactive medulla was crushed and injected under the skin of some dogs. The following day they were inoculated with a medulla that had been drying thirteen days, and so on, using that of increased virulence until the medulla used was of a rabbit dead the same day. The dogs might now be bitten by rabid dogs or subjected to the intracranial inoculations of the deadly virus; they resisted both. On Monday, July 6th, 1885, a little boy accompanied by his mother entered the laboratory, the boy had been terribly bitten by a mad dog several days before. Pasteur was convinced that the boy's only chance for life was through his attenuated virus treatment. It was a very slight operation, a more injection into the side of a few drops of the liquid prepared from the fragments of the medulla oblongata; these were repeated each day by using a stronger virus, until the fresh virus was injected, no signs of hydrophobia appeared. The first human life had been saved by what is known today as the pasteur treatment. In the weeks and months following hundreds were given the treatment with equal success, an immense building known as the Pasteur Institute was erected in Paris, entirely by public subscription where the work could be carried on. Pasteur had accomplished the great aim of his life.

In 1894, in the midst of his activities, Pasteur suffered an attack of uraemia. The vigor of youth was no longer able to aatisfy the demands of a fatigued mind and diseased

body. It was evident to his friends that the parting of the ways was near at hand, and though dawn had broken over a new area in médicine- made possible by the wonderful Pasteur - the shadows of evening were no less apparent in his life. The hand once strong and firm, the eyes once clear and discerning, could no longer bid obedience to the mind; the life that had given to the worls its first insight into the field of Bacteriology was soon to live only in memory of the thousands benifited by his discoveries. The genius that had saved France from a national calamity and many of its people from ruin was destined to survive not in France alone, but in every corner of modern civilization.

And so, while the visions of his life's work passed before him, and surrounded by his children and grandchildren, he passed away September 28, 1895, amid the softly fluttering leaves of early Autumn, which seemed

to nestle like the tear drops of a nation over the simple sod that marked his last resting place.

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